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BUREAU OF SPORT FISHERIES AND WILDLIFE

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THE

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TROUT HEPATOMA -- A PRELIMINARY REPORT

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and
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FISH PATHOLOGY AND ITS ROLE IN FISH CULTURE were brought into prominence in the spring of 1960 by the disclosure of a high incidence of hepatomas in hatchery-reared rainbow trout. The current problem came to light as the result of a routine inspection of live trout shipments at a California border fish-disease checking station. This service is performed by personnel of the California Department of Fish and Game to preclude the introduction or further spread of communicable fish diseases into California watersheds. Collaborative studies which followed revealed the nationwide distribution of the disease. This unusual disease soon attracted the attention of the Bureau of Sport Fisheries and Wildlife, the Food and Drug Administration, Public Health Service, and several western State health and conservation agencies.

On July 27, at Boise, Idaho, representatives of Federal and State health and conservation agencies met for the purpose of considering this outbreak of hepatoma. The representatives of the States issued the following statement:

> After consultation with competent State and national medical authorities, we conclude that the reported increased occurrence of hepatoma in trout may have some medical significance but that it poses no known problem to human health.
>
> We agree that this and related problems need further study. We intend jointly and in keeping with our responsibilities to undertake, support, and pursue a coordinated research program directed toward the clarification of the causative factors of hepatoma in fish and their possible implications as they are related to public health and to fishery management.
>
> We intend to pursue this study diligently and in every practical manner, and to keep interested organizations and the general public currently and completely informed.

Review

This condition has been reported in the literature for some time. In the British Isles, Haddow and Blake (1933: 43, 44) mentioned "Hepatoma in a rainbow trout" and noted that it "occurred in a group of 116 trout-farm fish (Salmo fario, S. irideus, S. fontinalis) found dead."

In the United States, Nigrelli (1954) mentioned the occurrence of hepatoma in a rainbow trout and published a photograph of it. Later, Nigrelli and Jakowska (1955) reported as follows:

> A comparatively large number of deaths in a single lot of 600 3-year-old trout occurred in a Pennsylvania hatchery. . . . In view of the controlled conditions under which these trout are raised and kept, hereditary and nutritional factors are suspected.

The disease was also observed in Italy in 1952 and reported by Cudkowicz and

Scolari (1955). The following is a translation of the Italian summary:

> The authors have described the general aspect, microscopic and macroscopic, the geographical distribution, and the diffusion of a primary liver tumor in hatchery rainbow trout. The neoplasm appeared at first as a sporadic disease among older trout which had been used for reproduction in some hatcheries of northern Italy; after a few years it reached epizootic proportions with an incidence sometimes of more than 50 percent.
>
> The tumor is of the hepatocellular type with trabecular and cirrhotic structure. The colangiomatous and colangiocarcinomatous areas are rare. Metastases at a distance have been observed in the excretory and hemopoietic part of the kidney, in the heart, the gastric wall, and the pyloric caeca. No hepatic parasites or cellular inclusions have been found. Attempts to transplant the tumor experimentally into healthy trout of the same race through a transplantation of fresh fragments, injections of acellular homogenates, and prolonged cohabitation of sick animals with healthy ones have not shown any positive results.
>
> The authors hold that the increased cancerous morbidity of the strain of rainbow trout raised in Italy is due to hereditary factors which were selectively developed in the course of a long series of inbreeding, which conditioned a particular lability of the hepatic cell with regard to the oncogenic stimuli. The trauma produced on the liver during the manual stripping of the gonads and the hemorrhagic extravasations which result from this could represent the external agent, realizing the neoplastic potentiality of the liver through proliferative processes of the regenerative nodular hyperplasia which have been induced in that way.

During the current investigation, consultation with others engaged in the field of fisheries indicated that hepatoma was not a new disease in the West. J. H. Wales observed and described this condition in rainbow trout in California in 1937 (unpublished records and personal communication, 1960). He wrote:

> It appears to have occurred only in the selected, fall-spawning stock taken to the Hot Creek Hatchery, California, from the U. S. Fish Hatchery, Springville, Utah, in 1933. The hepatoma first came to my attention at the Hot Creek Hatchery in 1937, at which time this particular brood stock was four years old; the estimated incidence was 50%. Hepatoma was next noticed at the Mt. Shasta Hatchery, California, in November 1939 among four-year-old rainbow trout which had been taken there from the Hot Creek Hatchery two years previously. Here the incidence of hepatoma was thought to have been considerably lower than at the Hot Creek Hatchery. Prior to the time that this strain of rainbow was brought to Mt. Shasta, a large brood stock of steelhead rainbow was kept at that hatchery. There is no evidence that these fish had any hepatoma; this stock was from wild Klamath River steelhead rainbow and had not been selected. Hepatoma appeared to have been present among the Hot Creek Hatchery fish from 1937 to 1940 and among the Mt. Shasta Hatchery fish from 1939 to about 1941.

It is interesting to note that hepatoma was observed in Idaho in about 1953. James Ellis (personal communication, 1960) found this condition present among his 1- to 2-year-old rainbow trout. They had been reared on diets containing fish meal, cottonseed meal, meat meal, and dried vegetable products, all in pellet form. A change in diet was made, after which the hepatoma no longer occurred.

Present Observation

The disease, as presently observed in rainbow trout (Salmo gairdneri) and cutthroat trout (S. clarki), is characterized by the presence of white nodules--varying in number and ranging from 1 to 50 millimeters or more in diameter--on the liver. In an advanced case of the disease, as shown in figure 1, there may be a bulging of the abdominal wall of the fish because of obvious tumors within. The arrows in figure 2 indicate the rather large tumors

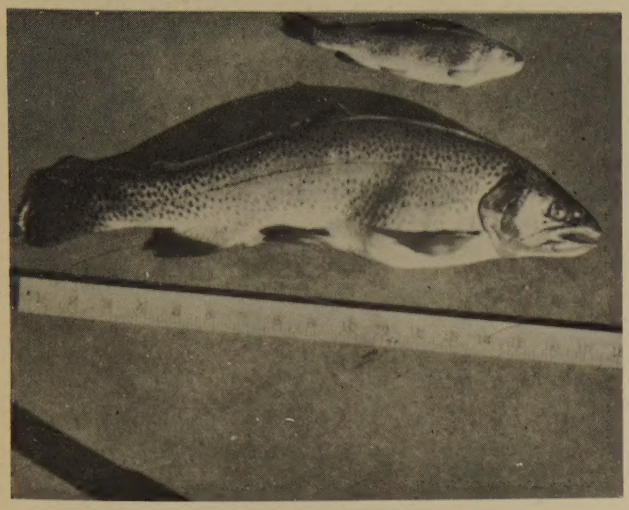

FIGURE 1.-- Adult rainbow trout with the abdominal wall bulging from obvious tumors within.

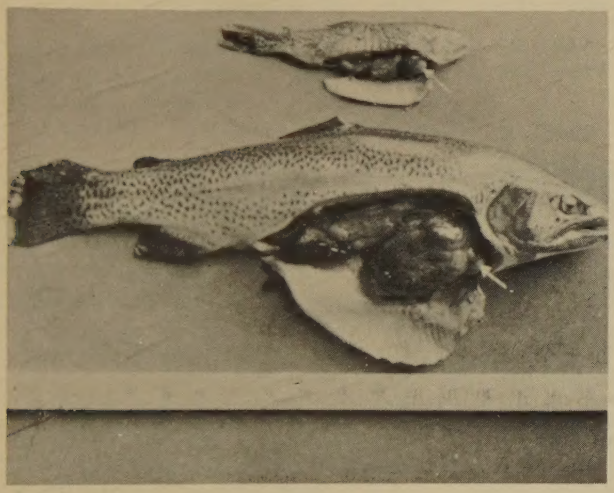

FIGURE 2.--The adult rainbow trout of figure 1, with the abdominal wall removed to show the hepatomas.

of the livers of the rainbow trout shown in figure 1. Nodules or whitish areas on fish livers (figure 3) do not obviate the possibility of the presence of other affections such as parasitic, protozoan, bacterial, or fungal infections. A microscopic examination is necessary, therefore, to identify definitely the nature of the nodule.

Microscopically, our findings appear to be very similar to those reported by Cudkowicz and Scolari (1955). All stages from adenomalike nodules to advanced carcinoma were observed.

Many of the grossly "normal" livers are found to have early stages of hepatoma. The tumor cells are generally similar to the surrounding normal cells, except for their stronger affinity to the basophilic stain as shown in figure 4. These areas may easily be mistaken for normal tissues (see figure 5) if examined separately. In fatty livers, which are often found in many of the domesticated fish, these islands of neoplastic cells are quite conspicuous because of their deficiency of fat vacuoles. They also lack the capsules, the fibrous membranes, which are generally associated with benign tumors.

The advanced stage of these tumors exhibits pathology which is more characteristic of a malignancy. Although mitotic figures are infrequent, loss of normal cellular differentiation and architecture is noted (figure 6). Necrotic and hemorrhagic areas, as well as abundant connective tissue areas, are also present. Metastases are sometimes found in the kidneys, and on one occasion a metastasis was observed in the spleen.

No metastases have been noted in the heart, gastric wall, or pyloric caeca,

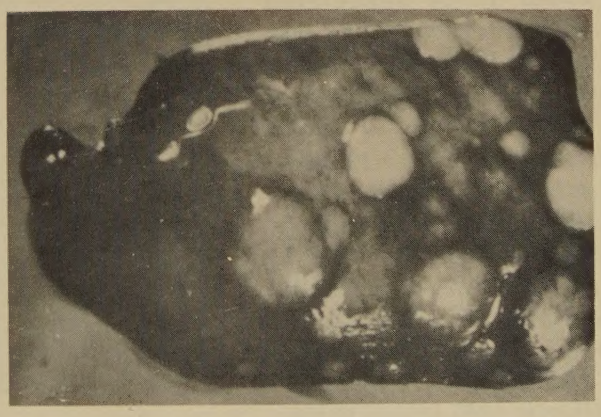

FIGURE 3.--Adult rainbow trout liver with nodular growth, hepatoma.

as was reported by Cudkowicz and Scolari (1955).

The etiology of trout hepatoma is a problem of serious concern to private, State, and Federal fish-rearing establishments and to the manufacturers of feeds for fish. The cause of the liver tumor must be known in order that intelligent recommendations for its cure, if possible, and its prevention may be made. Hepatoma in fish might be caused by hereditary, environmental, biological, or nutritional factors. There has been much discussion centering around heredity, but this factor and the environment seem to be only remote possibilities inasmuch as cases of hepatoma have occurred among many strains of trout in the Western States, as well as in the East, and under various environmental conditions. No organisms have been found to be associated with the tumor, nor has an infectious nature been demonstrated.

A nutritional basis for hepatoma carries greatest credence at this time; prevalence of the tumor appears to be correlated with the advent of fish meals and pelleted fish foods. There is some evidence that the development of hepatomas may be correlated with the

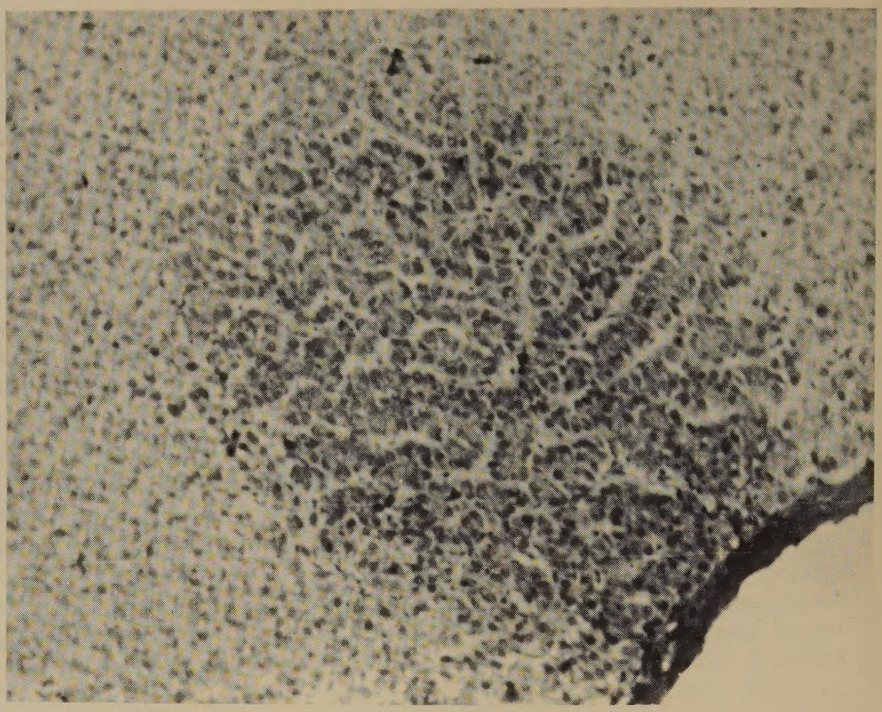

FIGURE 4.--Early stage of hepatoma, showing densely stained nodule from a yearling rainbow trout. Hematoxylin and eosin. X 200.

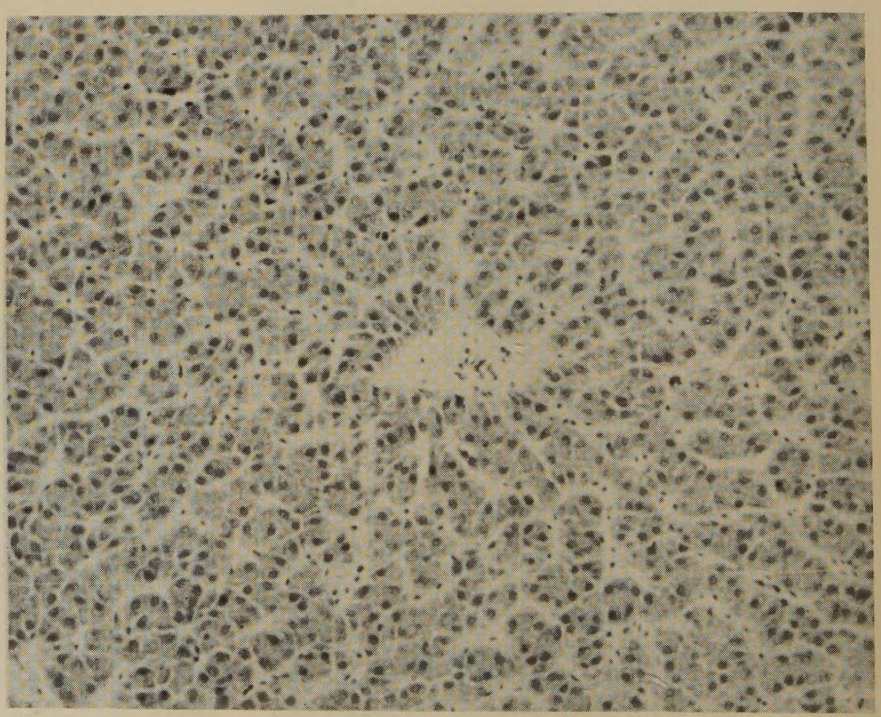

FIGURE 5.--Normal liver from yearling rainbow trout. Hematoxylin and eosin. X 200.

use of certain foods. Whether the cause is a deficiency, an imbalance, an excess of some compound, or the presence of some noxious agent is yet to be determined. This probably will be accomplished when sufficient numbers of fish have been fed a variety of diets containing definite known ingredients and the resulting information has been analyzed.

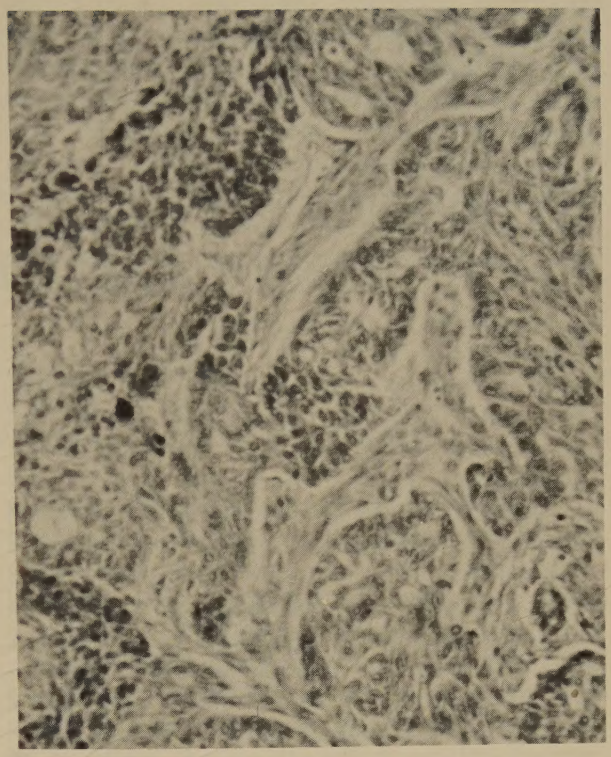

FIGURE 6.--Advanced stage of hepatoma, showing abundant connective tissue from a 16-month-old rainbow trout. Hematoxylin and eosin. X 200.

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NORMAL BEHAVIOR OF CHINOOK SALMON FINGERLINGS

At the Salmon-Cultural Laboratory, wild and hatchery chinook salmon fingerlings were observed for 30-minute periods in a tunnel in which water velocities were from 0.4 to 1.8 feet per second.

Initially the stop screen at the head of the tunnel was made of 0.25-inch hardware cloth. When the water velocity neared 1.5 feet per second, some fish--both wild and hatchery--would swim forward and cling to the screen by the teeth of the lower jaw. They hung on and maintained themselves with little effort. After this screen was replaced with a perforated vertical-slot screen, they continued--with much less success--to try to attach themselves to it. From this we assume that fingerlings normally maintain themselves in fast currents by clinging to stationary objects.

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COMPARISON OF VARIOUS METHODS OF DETERMINATION OF HEMOGLOBIN IN TROUT BLOOD

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HEMATOLOGICAL EXAMINATION is one of the most valuable of all laboratory diagnostic aids in human and veterinary medicine. Modern hematological methods are rapidly gaining recognition in fishery research and in practical fish culture. The purpose of the experiments described here was to check the accuracy and the dependability of the standard methods of hemoglobin determination when applied to blood of trouts. The blood of fish differs in many respects from that of mammals; therefore, some modifications in techniques or recalibration of instruments may be necessary to achieve the same degree of accuracy with the hematological methods when they are applied to fish blood.

In our review of the published information on the determination of hemoglobin in fishes, only those papers which were available to us in the original form will be mentioned. Data presented by Hall, Gray, and Lepkovsky (1926), Adrianow (1936), Smith, Lewis, and Kaplan (1952), and Dombrowski (1953) are expressed as percentages of hemoglobin concentration in normal human blood. For this reason, these figures require recalculation in order to conform to the presently accepted practice of expressing the quantity of hemoglobin in grams per 100 milliliters of blood. Field, Elvehjem, and Juday (1943) determined the hemoglobin in the blood of carp and brook trout. Suyehiro (1958) determined the hemoglobin, hematocrit, and number of erythrocytes in carp. Gelineo and Gelineo (1955) used the cyanhematin method for the quantitation of hemoglobin in 12 species of freshwater fish. Kisch (1949a, 1949b) determined the acid hematin hemoglobin in a large number of marine teleosts and selachians. Phillips (1958) and Snieszko (1960) determined the hemoglobin in brook trout, brown trout, and rainbow trout. The quantities of hemoglobin reported by Schumacher (1958) were somewhat lower than could be expected from the red cell counts.

The most detailed hematological examination of marine fishes representing Teleostomi, Elasmobranchii, and Cyclostomata was reported by Wintrobe (1934). In these studies hemoglobin was determined by means of a hemoglobinometer calibrated by the Van Slyke method; therefore Wintrobe's results must be considered as being very reliable. Wintrobe also found a very good correlation between the hemoglobin concentration, red cell count, and hematocrit in all vertebrates examined.

Data on quantitation of iron in fish blood were reported by Hall (1930), Hall and Gray (1929), Phillips and Brockway

---

Mr. Larsen was a trainee at the Eastern Fish Disease Laboratory during 1959-60 and is now attending the In-Service Training School at Marion, Alabama. After July 15, 1961, Mr. Larsen will be stationed at the New London National Fish Hatchery, New London, Minnesota.

(1958), Scholander and Van Dam (1957), and Shell. 1/

Hemoglobin and iron were simultaneously determined in the blood of some fish species by Hall, Gray, and Lepkovsky (1926), and by Saito (1954a, 1954b). Salmonids were not included.

Black (1955) determined hemoglobin photocolorimetrically as acid hematin in six species of fresh-water fish. Most of the values found were in the range of 10 to 11 grams of hemoglobin per 100 milliliters of blood. Such high values could be expected with this method if samples of acid hematin were not centrifuged before reading. Schiffman and Fromm (1959) used the same method for determination of hemoglobin in rainbow trout (Salmo gairdneri). As they centrifuged the acid hematin samples before reading, their figure of 6.51 grams was too low. This could be expected from findings presented in this paper. Hiestud (1951) determined hemoglobin as acid hematin in largemouth bass (Huro salmoides), again using the acid hematin method without centrifugation, and obtained average values of 10.33 grams of hemoglobin. Hemoglobin concentration in smallmouth bass (Micropterus dolomieu) as calculated from blood iron was 4.9 grams on the average. 1/ Haws 2/ used the oxyhemoglobin method for determination of hemoglobin in catfish; therefore his findings of 6.6 to 6.9 grams of hemoglobin can be compared with our results.

It is well established that in the blood of healthy vertebrates there is a close correlation between the number of erythrocytes, hematocrit value, and hemoglobin content. In dogs, for example, the mean erythrocyte number could be determined with a high degree of accuracy from a single hematocrit (Uglialoro and Alder 1957). Recently a similar relationship was found in trout (Snieszko 1960). Regression analysis of hemoglobin and hematocrit determinations which included simultaneous readings from 743 spring chinook salmon (Oncorhynchus tshawytscha) has given a correlation coefficient of 0.9238. 3/ In anemic salmon the relative decrease of hemoglobin was greater than that of hematocrit.

Generally, hemoglobin in human blood is determined by either the acid hematin method, the cyanmethemoglobin method, or the oxyhemoglobin method. Their descriptions can be found in any textbook of hematology (Wintrobe 1956) or clinical laboratory methods (Hawk, Oser, and Summerson, 1954). Apparently all these methods can be used for hemoglobin determination in mammals. Other vertebrates, including fishes, have nucleated erythrocytes which may affect the results. For example, if the acid hematin method is used for determination of hemoglobin in bird blood, a correction factor is recommended (Coffin 1945).

It is generally accepted that the most reliable methods for the determination of hemoglobin are based on the oxygen capacity or iron content. The error caused by iron content of serum is negligible (Hawk, Oser, and Summerson, 1954; Seligson 1958).

Results of iron determination in the blood of fishes were reported by a number of the authors mentioned previously. As there are several methods of spectrophotometric determination of hemoglobin, employing different derivatives of hemoglobin, Matsuura and Hashimoto (1958) checked the accuracy of some of these methods when applied to fish blood. These authors made their determinations with crystalline hemoglobin isolated from blood of three species of fish and horse blood. They found that the hemoglobin extracted from the blood of three species of fish and the horse was of a uniform nature and produced identical results with the same

1/Shell, Eddie Wayne. Chemical composition of the blood of smallmouth bass. Ph.D. thesis. Cornell University, Ithaca, New York. 1959.

2/Haws, Travis Glen. Some aspects of the hematology of two species of catfish in relation to their habitats. Ph.D. thesis. Purdue University, Lafayette, Ind. 1959.

3/Information received in a private communication from Mr. C. M. Whitomore of the Oregon Fish Commission.

method of spectrophotometric analysis. These results confirmed earlier findings which indicated that hemoglobin is chemically very uniform among all vertebrates. The hemoglobin molecule contains four atoms of iron and has a molecular weight of close to 67,000 (Fruton and Simmonds 1953). The iron content of hemoglobin is given as 0.34 percent. There is no information which would indicate that the iron content of the hemoglobin in fish is different from that in higher vertebrates; therefore the iron content of fish blood can be used as a reliable and accurate method of determining hemoglobin. The quantity so obtained can be used as a standard for evaluation of the direct colorimetric or spectrophotometric methods. This is important because fish-blood hemoglobin standards needed for calibration of instruments are not available commercially.

The experiments described here were performed in order to evaluate the relation between the iron contents and hemoglobin (as determined by other methods) in trout blood, and to use this information for calibration of instruments for colorimetric or spectrophotometric determination of hemoglobin by standard methods. We are not aware of any published report on simultaneous determination of iron and hemoglobin, by several methods, in trout blood.

Methods

For all the tests described here, blood was obtained from yearling brook trout (Salvelinus fontinalis), brown trout (Salmo trutta), and rainbow trout (Salmo gairdneri). For comparison, determinations were also made with human blood. Blood from trout was collected by severing the caudal peduncle; human blood was obtained by venipuncture. All blood was treated with heparin as an anticoagulant. Fourteen fish samples (six rainbow trout and four each of brook and brown trout) and two human blood samples were analyzed for:

1. Acid hematin hemoglobin
2. Cyanmethemoglobin
3. Oxyhemoglobin
4. Iron, by the Wong method (Hawk et al. 1954) with modification (Seligson 1958).

All determinations were made in a Bausch and Lomb "Spectronic 20" spectrophotometer, and all procedures (unless indicated otherwise) were as described by Hawk, Oser, and Summerson (1954). The calibration manual furnished with the instrument was followed for minor details of general operational procedures.

The accuracy of the instruments and of the methods was checked by (1) making complete duplicate hemoglobin determinations on two different samples of human blood and complete duplicate determinations on one rainbow, one brown, and one brook trout; (2) making all iron determinations in duplicate; and (3) determining iron and hemoglobin in duplicate by all methods used here on four dilutions of rainbow trout blood.

The method first used to determine iron was that of Wong (Hawk, Oser, and Summerson, 1954). Digestion, however, was difficult, and often incomplete, on fish blood. Therefore, a modification was introduced, as suggested by Hainline (Seligson 1958). This involved the use of boiling nitric and sulfuric acids. Rather than use a multiple dilution technique, a 0.5-milliliter sample of blood was pipetted into 2.0 milliliters of deionized, distilled water in a 25-milliliter Erlenmeyer flask. To this were added 2.0 milliliters of reagent-grade concentrated nitric acid and 2.0 milliliters of chemically pure sulfuric acid. The contents were then boiled gently under a hood on a thermostatically controlled hot plate to dense fumes of sulfur trioxide.

The digestion process took about 30 minutes. At this time digestion was so complete that the necessity for using a sodium tungstate precipitating process and subsequent filtration was eliminated. The sample was cooled, and determination then proceeded in accordance with Wong's method.

The acid hematin method employed was similar to that of Cohen and Smith

(Hawk, Oser, and Summerson, 1954), except that 0.02 milliliter of blood was added to 5 milliliters of dilute hydrochloric acid. The acid hematin method displayed two distinct disadvantages. The acid hematin color develops slowly, requiring approximately 1 hour to reach full development; and the suspended cell nuclei impart a turbidity which gives a falsely high reading (Hawk et al. 1954) unless a correction factor is used (Coffin 1945). To eliminate this difficulty, a modification of the acid hematin method was tried: this involved centrifugation of the treated sample at 3,000 revolutions per minute for 5 minutes. It was expected that the clear supernatant would give a true hemoglobin reading. For both variations of this method the instrument was calibrated with an acid hematin hemoglobin standard that is available commercially.

The cyanmethemoglobin method was that described by Wintrobe (1956), with no modifications. However, with fish blood caution must be exercised that the small gelatinous mass which forms does not go into the cuvette. This method has the advantage of readily available stable reagents and standards. There is little danger from pipetting the reagents, as it would take a large volume of reagent to produce a serious toxic effect of cyanide in man (Wintrobe 1956). The instrument was calibrated with a cyanmethemoglobin standard that is available commercially.

The oxyhemoglobin method employed was almost identical to that described by Wintrobe (1956), the only modification being the addition of 0.02 milliliter of blood to 5.0 milliliters of ammonia water to keep the size of the sample consistent. The oxyhemoglobin method offers no difficulty with nucleated erythrocytes but in fish blood produces a gelatinous mass that results in a false reading if placed in the cuvette.

Quantitative data on hemoglobin values obtained by calculation of hemoglobin from iron were compared with figures obtained by the other methods used. The correlation existing between the values obtained by different methods and the correction factor needed to convert the values obtained by any of the methods used to those obtained by determination of total iron in trout blood was calculated by using equations for linear correlation of two variables (Croxton 1959: chapter 6).

Results

As shown in table 1, values obtained by all three methods of hemoglobin determination on human blood samples are approximately equal to the values calculated from the iron content. All individual determinations and means are given in order to facilitate the comparison of findings in this work with data reported by other investigators, as well as to facilitate the interpretation of any future results.

The greatest percentage variation in the duplicate iron determinations on any of the blood samples was 9 percent, which occurred once. The average percentage variation was 2.4 percent for all samples.

The duplicate uncorrected hemoglobin determinations had an extreme variation of 8 percent for the oxyhemoglobin values of one human blood sample. Average variation of duplicate determinations by all methods was 2.9 percent. To calculate percentage variation, the smaller value in duplicate determinations was used as the base. However, the classical clinical methods of determining hemoglobin in man are not applicable to fish blood unless inherent differences are adjusted. With adjustment, the standard methods evaluated are sufficiently accurate to determine hemoglobin in ordinary clinical work, as an accuracy of plus or minus 5 percent, or even 10 percent, is accepted as being satisfactory (Wells 1956). Hemoglobin determination on the diluted fractions showed that the deviation from the expected value was within the range of instrument accuracy for both the cyanmethemoglobin value (using the correction factor) and for the oxyhemoglobin method (table 2). Use of these two methods is, therefore, fully justified for severe anemic conditions.

Of the two methods used for the determination of iron, the original method of

TABLE 1.--Determination of hemoglobin in trout blood by three methods as compared with values obtained from hemoglobin calculated from the total blood iron

Source of blood	Acid hematin (Not centrifuged)	Acid hematin (Centrifuged)	Cyanmethemoglobin	Oxyhemoglobin	Hemoglobin calculated from total blood iron
	(Grams per 100 milliliters of blood)				
Human blood, No. 1----	14.7	14.4	15.3	16.6	15.6
2----	14.3	14.5	14.7	15.6	14.7
Rainbow trout, No. 1--	15.7	8.5	11.0	11.7	11.7
2--	15.3	7.8	10.4	11.7	10.6
3--	14.9	7.6	9.5	10.4	10.5
4--	12.0	7.6	8.7	9.0	8.8
5--	11.7	6.5	8.6	9.5	9.5
Mean----------	13.9	7.6	9.6	10.5	10.2
Mean value after correction[1]----	9.86	10.06	10.04	10.04	----------
Brown trout, No. 1----	12.3	5.1	8.0	8.5	8.5
2----	13.4	6.1	9.0	10.1	9.1
3----	12.0	7.2	8.5	9.3	8.4
4----	13.0	7.7	9.6	9.4	9.4
Mean----------	12.7	6.5	8.8	9.3	8.85
Mean value after correction[1]----	9.22	9.77	9.2	9.10	----------

(Continued)

TABLE 1.--Determination of hemoglobin in trout blood by three methods as compared with values obtained from hemoglobin calculated from the total blood iron--Continued.

Source of blood	Acid hematin (Not centrifuged)	Acid hematin (Centrifuged)	Cyanmethemoglobin	Oxyhemoglobin	Hemoglobin calculated from total blood iron
	(Grams per 100 milliliters of blood)				
Brook trout, No. 1	16.2	[2] 3.8	9.3	10.7	10.2
2	14.1	6.1	9.3	10.4	10.0
3	14.9	7.4	10.1	11.7	10.4
4	14.9	6.9	10.4	10.9	10.9
Mean	15.0	6.05	9.8	10.9	10.4
Mean value after correction [1]	10.45	9.65	10.25	10.36	----------
Correction factor [3]	(x0.534) + 2.44	(x0.266) + 8.04	(x1.02) + 0.25	(x0.785) + 1.80	----------
Mean value for all trout after correction	9.8	9.8	9.8	9.8	(Mean value) 9.8
Average percent of difference of all individual corrections	0.37	0.81	0.34	0.35	----------

[1] These figures were obtained by using the correction factor.

[2] This figure was eliminated from calculations of means because it was obviously out of range as the result of a technical error in the determination of hemoglobin.

[3] This correction factor was calculated by the method of linear correlation of two variables and is used to convert the hemoglobin values determined from acid hematin, cyanmethemoglobin, and oxyhemoglobin to the values obtained by calculation of hemoglobin from the total iron in trout blood. For example, the correction for mean acid hemoglobin is: 13.9 x 0.534 + 2.44 = 9.8.

TABLE 2.--Duplicate determinations of hemoglobin by three standard methods and iron in serial dilutions of a single sample of rainbow trout blood

Hemoglobin level, original samples	Calculated from iron	Acid hematin		Cyanmethemoglobin		Oxyhemoglobin
		Uncorrected	Corrected	Uncorrected	Corrected	Uncorrected
		(Grams per 100 milliliters of blood)				
100 percent	9.0, 9.2	12.7, 11.6	9.2, 8.6	8.2, 8.2	8.6, 8.6	8.8, 8.5
75 percent	1/ 6.8	8.5, 8.5	7.0, 7.0	5.7, 5.7	6.0, 6.0	6.4, 6.4
50 percent	1/ 4.5	6.5, 6.1	5.9, 5.7	3.9, 3.9	4.2, 4.2	4.3, 4.4
25 percent	1/ 2.3	2.8, 2.8	3.9, 3.9	1.6, 1.6	1.9, 1.9	1.9, 1.9

1/ Determined on the basis of dilution of original sample.

Wong was found unsatisfactory as used here with trout blood. The digests were turbid, a condition which necessitated filtration. This, in turn, could lead to errors, as some of the iron could be absorbed by the precipitate.

The acid hematin method without correction was found unsatisfactory for fish blood because nuclei of erythrocytes remained in suspension. This is apparent from table 1, which shows that the uncorrected values obtained by the standard acid hematin method were approximately 50 percent greater than those calculated from iron determinations. The factor for correcting standard acid hematin values for fish blood was

0.534 x A-H Hb value + 2.44,

where A-H Hb = acid hematin hemoglobin. The corrected values then showed a standard deviation of 3.8 percent and a range of 0 to 8.8 percent as compared to the values calculated from the iron determinations. At extremely low hemoglobin levels (table 2), the adjustment factor does not give good results because of dispersion of the suspended nuclei. This method is therefore considered the least desirable of the three.

The centrifuged acid hematin method yielded poor results. After centrifugation the sediment was dark in color, indicating possible absorption of some hemoglobin. The uncorrected values were consistently below the values that were calculated from the iron content. Even the corrected values showed a standard deviation of 8.3 percent and a range of 3.0 to 19.0 percent. The correction factor for this method is therefore completely unreliable.

Results from the cyanmethemoglobin method were generally slightly lower than the hemoglobin values calculated from the determination of total iron. Therefore, a correction factor was considered necessary. This factor was determined to be

1.02 x CM-Hb value + 0.25,

where CM-Hb = cyanmethemoglobin. The corrected hemoglobin values displayed a standard deviation of 3.5 percent and a range of 0 to 7.4 percent from the calculated values. This method is, at present, the method of choice, as the reagents are stable, easily obtained, and give consistent results.

The oxyhemoglobin determination also appears to be fairly reliable, although the uncorrected value is generally slightly higher than that calculated from total iron. A correction factor was calculated and applied to the unadjusted results. The corrected values then showed a deviation of 3.6 percent and a range of 0 to 8.3 percent from the calculated values. Because the uncorrected values showed a deviation of only 4.2 percent and a range of 0 to 12.5 percent, a correction factor was not considered advisable for this method.

The comparison of different methods of hemoglobin determination was made with blood from three species of trout.

Although the total number was sufficient to determine the correlation between the methods, the small numbers of trout of each species were not sufficient to establish significant hemoglobin values for each species. The different species were used to eliminate the possibility that species of trout might interact with methods in the determination of hemoglobin content. An interpretation of the analysis of variance, factorial design, has satisfied the authors that no such effect existed.

Using a factor (if necessary), hemoglobin standards, and a suitable electrophotometer or colorimeter, it is thus possible to determine acceptably accurate hemoglobin values for trout blood. It must be stressed that all instruments must be calibrated in terms of a standard.

Any small, portable hemoglobinometer can and should be calibrated with the aid of an electrophotometer. This can be done simply by first calibrating the electrophotometer with a known standard, such as the cyanmethemoglobin standard already mentioned. Directions for calibration accompany the standard. Then several samples of fish blood should be examined, both electrophotometrically and with the pocket instrument. The equation that is applicable to all hemoglobin-determination methods is:

$$\frac{\text{Hemoglobin value of unknown determined electrophotometrically} \times \text{standard method factor}}{\text{value obtained with pocket instrument}} = \text{Factor for pocket instrument.}$$

This factor can then be multiplied by the value obtained with the pocket meter to give a close approximation of the hemoglobin value of the fish-blood samples being examined. In this way, readings taken with different instruments and in different regions will have a standard value and can be compared.

Previously, differences in instruments and methods provided such a magnitude of variation in the values that any correlation between different sources was impossible. Through proper standardization and use of instruments, the reporting of hemoglobin values in fish should prove itself a more valuable tool in diagnostic services.

Summary

Quantitative determination of hemoglobin in fish is complicated by the presence of nuclei in erythrocytes. This requires standardization of methods by the determination of iron or oxygen capacity of total blood. In this experiment, iron was determined in trout and human blood by the Hainline modification of the Wong method. The quantity of hemoglobin calculated from iron was used to obtain correction factors for quantities obtained by the methods of acid hematin, cyanmethemoglobin, and oxyhemoglobin. The cyanmethemoglobin method was found the most reliable; the oxyhemoglobin method was next best. The acid hematin method gave erratic results because of suspended nuclei of erythrocytes.

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Between July 1, 1958, and June 30, 1959, a total of 19,914,021 fishermen in 50 States spent $50,374,832 for 22,861,880 fishing licenses, tags, permits, and stamps required by State fish and game departments to fish legally for sport or recreation.

A Norwegian trout hatchery uses a number of fiberglass tanks (about 20 by 20 feet) set on concrete rings, with a drain in the center and hinged standpipes controlling the water level just outside. Water is brought in by plastic pipe. These tanks are durable, require no maintenance, and are easily transported and set up. When the water in one location becomes too warm or too cold or threatens to dry up, the tanks are moved to another location and set up in a day.

UTILIZATION OF MACROCRUSTACEANS FOR FOOD BY FRESH-WATER FISHES IN LOUISIANA AND ITS EFFECTS ON THE DETERMINATION OF PREDATOR - PREY RELATIONS

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THE LARGE PREDACEOUS FISHES IN FRESH-WATER FISH POPULATIONS, and especially those in warm-water fish populations, are usually considered as being piscivorous. This has led to the development of various predator-prey ratios in an attempt to describe and measure the relations between predaceous fishes and their prey fishes. Probably the most widely known are Swingle's (1950) F/C ratio, in which F equals the total weight of all forage fish and C equals the total weight of all carnivorous or piscivorous fish in a population, and his Y/C ratio, in which Y equals the total weight of those individuals in the forage group that are small enough to be eaten by the average adult in the carnivorous group of fish and C equals the weight of the carnivorous fish in the populations. Swingle used these ratios primarily with bass-bluegill combinations in ponds, but he advocated their use for natural populations. Lambou (1959) used the following ratios in describing predator-prey relations in backwater lakes in Louisiana: (1) pounds of non-predaceous fish per pound of predaceous fish, and (2) pounds of total fish under 5 inches per pound of predaceous fish over 5 inches.

Some of this work was undertaken with Federal Aid in Fish Restoration funds, as a part of Dingell-Johnson Project F-1-R, Louisiana.

As the use of predator-prey ratios has become widespread among biologists, it is worthwhile to evaluate some of the factors which affect the validity of such ratios. When Surber suggested a standardized method of reporting fish-population data for reservoirs (in press), he summarized some of the problems that must be solved in order to be able to determine predator-prey ratios more accurately.

Fish populations have been sampled extensively by rotenone poisoning in Louisiana, as well as in the whole Southeast. Usually crustaceans cannot be sampled by rotenone poisoning. Therefore, in determining predator-prey relations based on such sampling data, it is not possible to include the amount of available forage other than fish (e.g., macrocrustaceans) in the predator-prey ratios. If macrocrustaceans are important in the diet of the large predators in a fish population, predator-prey relations determined by rotenone poisoning will be biased. It is important, therefore, to know to what extent large crustaceans are used for food by predaceous fish.

Lambou (1959) found that the backwater lakes contained 0.2 pound of fish under 5 inches for each pound of predaceous fish over 5 inches. It did not seem reasonable that the predaceous fish in the backwater lakes would support themselves on considerably less available forage (by weight) than they themselves made up in the population, but it was concluded that swamp

crayfish (Procambarus clarki) were important in the diet of these large predators. However, no supporting data were presented at that time.

Material and Methods

Fish stomachs were collected from three areas in Louisiana: Howze Beach, The Flats, and Bonnet Carre Spillway Canal (see figure).

Howze Beach is a series of shallow, brackish marsh ponds along the northeastern shore of Lake Pontchartrain, an estuarine lake with a surface area of 635 square miles. At one time Howze Beach was agricultural land. Farming proved unprofitable and was abandoned, however, and the levees which at one time prevented water from entering Lake Pontchartrain have broken down. The area now consists of a system of ponds and canals which had been used to drain the land. The ponds range from a few inches to approximately 5 feet in depth, but the majority are 2 or 3 feet deep. Most of the canals are 6 to 8 feet deep.

Fluctuations in the salinity of the water from year to year and within years are considerable. During 1951-52 salinities of 1,848 to 5,148 p.p.m. were recorded. However, salinities were recorded only during five sampling periods, and it is believed that they exceeded this range in both directions.

Fish stomachs were collected from June 1951 through July 1952. Alligator gar (Lepisosteus spatula) and spotted gar (L. productus) were collected by fishing with a wire noose similar to that described by Gowanloch (1939). For bait, yellow bass (Morone interrupta) were tied to the noose with thread. Gars were also collected at night in shallow water by gigging. All were killed immediately after capture, and after each trip the stomachs were examined and the contents recorded.

Specimens of largemouth bass (Micropterus salmoides) and yellow bass were collected by me or by sport fishermen accompanying me. Largemouth bass were caught by casting with artificial baits, but yellow bass were caught by pole fishing

Location of sampling stations.

with shrimp as bait. All fish were killed immediately after capture. Most of the stomachs were examined after each fishing trip, but some were put in 5-percent formalin solution and examined at a later date.

Stolen bait was found in some of the yellow bass and gar stomachs. Shrimp was the only bait possible for the yellow bass to steal. If only a small piece of shrimp appeared in the stomach and little digestion had taken place, it was considered stolen bait; whereas if the shrimp was found whole (head, body, and tail present) or in an advanced stage of digestion, it was recorded as natural food. In the case of gars, yellow bass was the only stolen bait possible. All of the yellow bass, when found in the stomach of a gar, had the thread used in fastening the bait to the wire noose still attached and therefore could be readily identified.

The Flats is a backwater lake in the flood plain of the Atchafalaya River. For a general description of Louisiana backwater lakes, see Lambou (1959). The water level in The Flats fluctuates extensively, depending upon the water level in the Atchafalaya River. Large expanses of land adjacent to the lake, though normally dry, are flooded during periods of high

TABLE 1.--Species collected, number of stomachs examined, and number and percentage of those containing food

Species	Place of collection	Total length (inches)	Total number of stomachs examined	Stomachs with food	
				Number	Percent
Alligator gar--	Howze Beach----------	33 - 73	54	30	55.6
Spotted gar----	Howze Beach----------	10 - 39	44	22	50.0
Yellow bass----	Howze Beach----------	4 - 12	273	167	61.2
Largemouth bass	Howze Beach----------	7 - 18	93	59	63.4
Largemouth bass	The Flats (1958)-----	9 - 18	67	28	41.8
Largemouth bass	The Flats (1959)-----	8 - 20	120	35	29.2
Spotted bass---	The Flats (1958-59)--	11 - 14	6	3	50.0
Blue catfish---	Bonnet Carre Spillway Canal.	> 14	20	20	100.0

water in the river. Most of the high-water stages occur during late winter, spring, and early summer; however, the depth and extent of flooding vary from year to year.

Specimens of the largemouth bass and spotted bass (Micropterus punctulatus) were collected from The Flats during the periods June through October 1958 and May through July 1959 by me or by sport fishermen accompanying me. Artificial baits were used. All fish were killed immediately after capture; the stomachs were examined after each fishing trip.

The Bonnet Carre Spillway Canal is south of Lake Pontchartrain, into which it enters. Dug to provide fill for the guide levees of the Bonnet Carre Spillway, the canal is essentially a fresh-water habitat; the water level fluctuates with the tides in Lake Pontchartrain. Stomachs of blue catfish (Ictalurus furcatus) collected during June 1954 while the fish population in the canal was being sampled by rotenone poisoning were examined.

Utilization of Macrocrustaceans for Food

Alligator Gar. --The stomachs of 54 alligator gar from Howze Beach were examined (table 1). Blue crabs (Callinectes sapidus) were found in 87 percent of the 30 stomachs which contained food (table 2). The blue crabs which could be measured were from 1 to 5 inches in carapace width. Darnell (1958) examined five alligator gar specimens (903 to 1,472 millimeters) from Lake Pontchartrain, and three of these specimens contained food. This included 65 percent large blue crabs and 35 percent striped mullet (Mugil cephalus), by volume. Darnell also stated that in addition to these five specimens, a large number of alligator gar were examined qualitatively in the field and all were found to be loaded with medium-sized and adult blue crabs.

Spotted Gar. --The stomachs of 44 spotted gar from Howze Beach were examined (table 1). Blue crabs were found in 55 percent of the 22 stomachs which contained food, and fishes were found in 50 percent (table 2). The blue crabs that could be measured were from 0.25 to 1.5 inches in carapace width. Of the fishes which could be identified, all were sunfishes (Lepomis spp. and/or Chaenobrytus coronarius). Darnell (1958) examined nine spotted gar specimens (405 to 555 millimeters) from Lake Pontchartrain, and seven of these contained food. This included 70 percent crabs and 24 percent fish, by volume.

Yellow Bass. --The stomachs of 273 yellow bass from Howze Beach were examined (table 1). Shrimp (Penaeus spp.; all or the majority believed to be the white shrimp, P. setiferus) were found in 31 percent of the 167 stomachs containing food, blue crabs were found in 29 percent, grass shrimp (Palaemonetes spp.) in 10 percent, and fishes in 5 percent (table 2).

TABLE 2.--Occurrence of specific food items in stomachs

Food item	Species:	Percentage of stomachs containing food items							
					Largemouth bass				
	Number:	Alligator gar 30	Spotted gar 22	Yellow bass 167	(Howze Beach) 59	(1958; The Flats) 28	(1959; The Flats) 35	Spotted bass 3	Blue catfish 20
Blue crab		86.7	55.0	29.0	55.9	----------	----------	-------	100.0
Swamp crayfish		----------	-------	------	------	85.7	54.3	100.0	-------
Shrimp		3.3	-------	30.7	25.4	----------	----------	-------	-------
Grass shrimp		----------	-------	10.1	6.8	----------	----------	-------	-------
Fishes		3.3	50.0	4.8	11.9	10.7	45.7	-------	-------
Unidentified fish		3.3	36.4	4.8	11.9	10.7	31.4	-------	-------
Sunfishes		----------	13.6	------	------	----------	8.6	-------	-------
Gizzard shad[1]		----------	-------	------	------	----------	2.9	-------	-------
Threadfin shad[2]		----------	-------	------	------	----------	2.9	-------	-------
Aquatic insects		----------	-------	2.4	6.8	----------	2.9	-------	-------
Adult dragonflies		----------	-------	------	------	3.6	5.7	-------	-------
Vegetation		----------	-------	.6	1.7	----------	----------	-------	-------
Unidentified objects		3.3	-------	4.2	1.7	----------	----------	-------	-------
Bait		13.3	-------	10.7	------	----------	----------	-------	-------

[1] (Dorosoma cepedianum). [2] (Signalosa petenensis).

The blue crabs which could be measured were less than 1.5 inches in carapace width. Darnell (1958) examined 27 yellow bass specimens (130 to 195 millimeters) from Lake Pontchartrain, and 18 of these contained food. This included 24 percent shrimp (schizopods, juvenal penaeids, Palaemonetes sp., and Macrobrachium ohione), 28 percent crabs (Callinectes sapidus and Rithropanopeous harrissii), and 35 percent fishes, by volume.

Largemouth Bass. -- The stomachs of 93 largemouth bass from Howze Beach were examined (table 1). Blue crabs were found in 56 percent of the 59 stomachs containing food, shrimp (Penaeus spp.; all or the majority believed to be the white shrimp) were found in 25 percent, fishes in 12 percent, grass shrimp in 7 percent, and aquatic insects in 7 percent (table 2).

The stomachs of 67 largemouth bass from The Flats were examined during 1958, and 120 largemouth bass stomachs from The Flats were examined during 1959 (table 1). During 1958 swamp crayfish were found in 86 percent of the 28 stomachs containing food, while fish were found in 11 percent, and adult dragonflies (order Odonata; suborder Anisoptera) in 4 percent (table 2). During 1959 swamp crayfish were found in 54 percent of the 35 stomachs containing food, fish were found in 46 percent, and adult dragonflies in 6 percent.

Darnell (1958) examined three largemouth bass specimens (175 to 209 millimeters) from Lake Pontchartrain and found that two stomachs contained food. Blue crabs formed 97 percent of the contents, by volume.

Spotted Bass. --Six spotted bass from The Flats were examined. Of these, three stomachs contained food (table 1). Swamp crayfish was the only food item noted.

Blue Catfish. -- The stomachs of 20 large blue catfish--collected while fish-population samples were being taken by rotenone poisoning in the Bonnet Carre Spillway Canal--were examined. Every specimen contained large numbers of small blue crabs. Usually fish collected by rotenone poisoning are not suitable for food-habit studies, as the large fish often feed on the smaller fish which show signs of distress from the rotenone before the larger fish are affected. However, because the concentration of rotenone used in the sampling (approximately 1 p.p.m.) apparently does not affect blue crabs and many of them were in advanced stages of digestion, it appears that the occurrence of blue crabs in the stomachs of the blue catfish was due to natural feeding.

Darnell (1958) found that macrocrustaceans (crabs, shrimp, etc.) and fish were important in the diet of large blue catfish (200 to 411 millimeters) in Lake Pontchartrain. Macrocrustaceans formed 8 percent of the volume of 15 blue catfish stomachs containing food (size range, 200 to 229 millimeters); isopoda and amphipoda formed 23 percent; mollusks, 43 percent; fish, 13 percent. Macrocrustaceans formed 17 percent of the volume of 23 blue catfish stomachs containing food (size range, 250 to 411 millimeters); isopoda and amphipoda formed 7 percent; mollusks, 12 percent; fish, 30 percent.

Channel Catfish. -- Darnell (1958) examined the stomachs of two large channel catfish (207 to 312 millimeters) from Lake Pontchartrain and found that they had consumed quantities of crustaceans (isopods, xanthid crabs, and others) as well as smaller amounts of fish, hydroids, and undetermined organic matter.

Difference in Occurrence of Swamp Crayfish in the Diet of Largemouth Bass in The Flats in 1958 and 1959

There was a considerable difference in the occurrence of swamp crayfish and fish in the diet of largemouth bass of The Flats in 1958 and 1959 (table 2). Swamp crayfish occurred in 24 stomachs and fish in 3 during 1958, whereas swamp crayfish occurred in 19 stomachs and fish in 16 during 1959. A chi-square test of independence was significant at a probability of less than 0.01 (chi-square equals 7.036 with 1 degree of freedom). Therefore, if it is assumed that the data contain no serious bias, a real difference exists in the proportion of swamp crayfish to fish in the diet of largemouth bass for the 2 years.

Before this hypothesis is accepted, possible sources of bias should be considered. The manner in which the largemouth bass were collected could be a source of bias. Collecting trips were made whenever the opportunity presented itself, and fish collected by sport fishing are surely a biased sample of the fish population. These factors could introduce bias into the data.

Largemouth bass collected during the various months were not equally represented in the samples taken during the 2 years: thus, 57 specimens were collected in June, 4 in August, and 6 in October of 1958; whereas 55 specimens were collected in May, 36 in June, and 29 in July of 1959. A careful examination of the empirical data did not demonstrate any correlation of stomach contents and time of year. Also, during 1958 many of the largemouth bass that I caught by sport fishing from The Flats prior to June were examined in the field. Even though the stomach contents were not recorded, gross observations did not demonstrate any noticeable difference in the relative importance of crayfish and fish in the diet for those caught prior to June and those caught after June.

I make no claim that the data presented show the exact relative proportion of crayfish to fish in the diet of the largemouth bass population in The Flats; however, because of these data, collateral information accrued, and other data to be presented later, I accept the hypothesis that there was a real difference between the 2 years. Undoubtedly a better design for collecting the data would have yielded more useful information.

Probably the reason for this difference in the food habits of the 2 years is the drastic reduction in the abundance of swamp crayfish in the Atchafalaya River basin during 1959. In normal years there is an extensive commercial fishery for swamp crayfish in the Atchafalaya River basin. No accurate records as to the size of this fishery are available, but Viosca (Anonymous 1959) reported that some 2 million pounds of swamp crayfish are landed at the small town of Henderson during a normal year. This is considerably above the total statewide production listed in various fishery statistical summaries. Even though no accurate statistics on the landings of swamp crayfish are available, there is no doubt that the reduction in their abundance during 1959 was real. This is attested by the fact that swamp crayfish were almost nonexistent in the markets during 1959 and by the numerous requests that the Louisiana Wild Life and Fisheries Commission received during 1959 for an investigation into the scarcity of swamp crayfish (Anonymous 1959). Viosca (Anonymous 1959) described this as "the recent crawfish catastrophe in the Atchafalaya Floodway."

If a reduction in the number of swamp crayfish forced the largemouth bass to turn to alternate food supplies, and the total amount of available forage was less during 1959 than during 1958, it would be expected that in these 2 years there would be a difference in the proportion of stomachs examined which contained food. During 1958, 42 percent of the stomachs that were examined contained food; during 1959, only 29 percent contained food. A chi-square test of independence was significant at a probability of less than 0.005 (chi-square equals 25.282 with 1 degree of freedom).

There was also a considerable difference in the size of the swamp crayfish found in the stomachs during the 2 years (table 3). During 1958 all swamp crayfish were in the medium and large size classification. However, during 1959, 12 were in the very small and small size classification, while only 7 were in the medium and large size classification. A chi-square test of independence between the proportion of very small and small to medium and large swamp crayfish in the stomachs was significant at a probability of less than 0.005 (chi-square equals 18.003 with 1 degree of freedom).

It was thought that there might be a difference in the number of swamp crayfish per stomach for the 2 years (table 3). A chi-square test of independence between the proportion of stomachs which contained 1 swamp crayfish to those which

TABLE 3.--Comparison of size of measurable swamp crayfish (total length of body excluding appendages), and of number of stomachs containing one or more swamp crayfish, in largemouth bass collected from The Flats during 1958 and 1959.

	Number for 1958	Number for 1959
Size of crayfish:		
Very small to small-------	----	12
Very small (<1.5 inches)	----	11
Small (1.5 - 3.0 inches)	----	1
Medium to large----------	24	7
Medium (3.0 - 4.5 inches)	20	6
Large (>4.5 inches)------	4	1
Stomachs with:		
1 crayfish----------	18	15
More than 1 crayfish-------	6	4
2 crayfish----------	4	2
3 crayfish----------	1	1
4 crayfish----------	1	----
5 crayfish----------	----	----
6 crayfish----------	----	1

contained more than 1 was not significant (chi-square equals 0.004 with 1 degree of freedom).

No weights of the largemouth bass were recorded; therefore I am unable to make any exact comparison of the condition of the fish for the 2 years. However, on the basis of gross examination of the fish, their relative condition was noted. All the largemouth bass examined during 1958, without exception, had large, noticeable deposits of fat around their viscera; but this condition was not noted on a single largemouth bass collected during 1959.

The data presented here tend to support the hypothesis that in normal years largemouth bass in backwater habitats similar to The Flats use mainly swamp crayfish for forage. In 1959, when largemouth bass in The Flats had to turn to foods other than the swamp crayfish, and forage was less abundant, the bass fed less often on forage of smaller size, with the result that the population was in poorer condition. This could have important effects on the largemouth bass population and on the sport fishery, though what these effects would be is not evident from this study.

Effects on the Determination of Predator-Prey Relations

The data presented show that macrocrustaceans are important in the diet of some of the large predaceous fishes that occur in some fresh-water populations in Louisiana. This does not mean that this is true for these species in all types of habitat in Louisiana. There is ample evidence in the literature that these species are piscivorous in many habitats.

The determination of predator-prey ratios such as those used by Swingle (1950) and Lambou (1959) will not be valid in habitats where macrocrustaceans are important in the diet of the large predaceous fish and where the sampling methods used do not sample the macrocrustaceans, i.e., unless some alternate scheme is used to estimate the macrocrustacean population. However, I do believe that the determination of such predator-prey relations is of considerable value where the large predaceous fishes are mainly piscivorous. Undoubtedly, much improvement needs to be made in the methods of measuring predator-prey relations. The work of Lawrence (1958) in determining the sizes of various forage fishes that largemouth bass can swallow is surely a right step in providing information needed for this purpose. More studies of this nature are urgently needed.

Acknowledgments

The data from Howze Beach were a portion of a thesis submitted to the Louisiana State University and Agricultural and Mechanical College, Baton Rouge, in partial fulfillment of the requirements for the degree of Master of Science, August 1952. The data from the other localities were collected while I was working on Louisiana's Dingell-Johnson Project F-1-R. I am indebted to Professors B. A. Bateman and L. C. Glasgow of the Louisiana State University School of Forestry for their direction and guidance on the Howze Beach portion of this study. I am also indebted to the many employees of the Louisiana Wildlife and Fisheries Commission who

assisted in the collection and analysis of the data and made many useful suggestions.

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SURVEY OF COTTIDAE IN JAPAN

"Cottidae (Pisces), Fauna Japonica" by Masao Watanabe, published by the Biogeographical Society of Japan (Tokyo News Service Ltd., Tokyo), has appeared as the first volume in a series--Fauna Japonica--in which leading Japanese taxonomists will describe the various animal groups of Japan.

Watanabe's survey of the Cottidae of Japan comprises scientific descriptions of the family, subfamilies, genera, and species.

The Cottidae have been reorganized and presented in 12 subfamilies instead of the 5 appearing in an earlier taxonomy. Changes in the arrangement and association of genera are largely due to new information on the morphology and osteology of Japanese fishes. The number of species has been enlarged to 74. Only Japanese synonyms are included, and these are minimal. The keys to the subfamilies, genera, and species are usually good, employing several characters.

The treatment given to each species includes a detailed discussion of its morphology, its color (based upon a single specimen), and its distribution in Japan. The painstaking effort with which data in this survey have been obtained is indicated by the fact that the 30 mensural characters tabulated for each of the 74 species are based on the examination of 5 to 50 specimens.

The illustrations are admirable. All species are depicted in 40 large, well-reproduced plates (15 of which are in natural color, the remainder in stippled line drawings). Text figures by the author show dorsal and ventral views of the head and peculiar characters.

---D. E. McALLISTER, National Museum of Canada, Ottawa, Ontario.

RESILIENCE OF A FISH POPULATION IN A STREAM IN PENNSYLVANIA

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IN THE LARGE NONGLACIATED AREAS of the eastern United States there are few natural lakes. Here the management of stream populations of fish becomes an important part of the program of furnishing recreation to sport fishermen. The fish taken by anglers include nearly all the game fishes common to the region, but the management of trout in small to moderate-sized streams has been emphasized by fish-management agencies. Six Mile Run, Warren County, in northwestern Pennsylvania is an example of such a stream.

For many years the management of these streams has consisted almost exclusively of stocking trout, with an occasional attempt to improve the environment by constructing low dams, deflectors, and various types of fish shelters. More recently, however, chemical toxicants have been used to eliminate or control large populations of unwanted species prior to stocking trout (Mullan 1956, Lennon and Parker 1959).

The objective of the present work was to eliminate a mixed fish population from Six Mile Run, prior to stocking it with brook trout only. The stream has a history of good brook trout fishing, but in recent years the brown trout and several nongame species have dominated the fish population. The original objective of killing all fish in the stream was not accomplished for several reasons. However, it was considered desirable to record the rapid expansion of the fish population after its severe reduction by rotenone. It is also important to point out the failure of a brook trout planting to compete successfully within such a rapidly expanding fish population. The recuperative ability or resilience of fish populations in suitable environments has been noted previously (Thompson 1944; Larimore, Childers, and Heckrotte, 1959), but this phenomenon continues to be underestimated by fish managers in their reclamation programs.

Prior Use of Rotenone in Streams

Berry and Larkin (1954) tested the toxicity of several concentrations of Fish-Tox, a commercial product containing an unknown percentage of rotenone, in a number of different stream situations in British Columbia. Although the temperature of the water, the amount of vegetation, and the amount of organic detritus present affected the toxicity of this product to the fish, concentrations of about 5 p.p.m. were necessary in many areas to assure a reasonable kill.

Lennon and Parker (1959) also investigated factors which affect the efficiency of eradicating a stream population composed of many different species of fish. After making a very careful survey of the stream flow, the fish species present, and the concentration and exposure time necessary to kill the various species, they

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were able to devise an elaborate procedure for applying the toxicant which was successful in killing all fish present. To kill some of the more resistant species, such as carp, a 6-hour exposure to a concentration of 1 p.p.m. of Pro-noxfish was necessary.

Methods of Treatment for Six Mile Run

Six Mile Run is a soft headwater tributary of the Tionesta River, draining a very small portion of the western slope of the Allegheny plateau. It is only a few miles in length and reaches a width of about 30 feet at its mouth. The stream volume fluctuates a great deal, from a few cubic feet per second in dry summer periods to hundreds of cubic feet per second during spring runoff. The bottom is composed of rounded stones, gravel, and sand, and has a very sparse growth of higher aquatic plants. The turbidity is usually low, and the water is only slightly colored.

Six Mile Run was treated with rotenone during a low-flow period in the fall of 1957. A party of five men applied the toxicant over six different sections along the stream, using a concentration sufficient to exceed 1 p.p.m. for the entire 3.5 miles treated. All backwaters, isolated pools, spring seepages, and small tributaries were also covered. In the vicinity of these application sites, the actual concentration probably exceeded 5 p.p.m. for short distances; but the exposure time at these higher concentrations was very short. Fish began dying within an hour after treatment began, and all fish were collected during the first day. No living fish were observed in the stream immediately after the treatment, but this should not be considered as conclusive evidence that all fish were killed.

Although the 1 p.p.m. concentration of rotenone used in Six Mile Run, together with a rather short exposure time, was somewhat less than the treatment shown to be necessary for complete kill of fish in streams, it was deemed inadvisable for public-relations purposes to use more of the toxicant and thus extend the fish kill unnecessarily beyond the mouth of the stream.

In the summer of 1958, limited sampling with electrofishing gear revealed the presence of many species of fish in addition to the stocked brook trout. Occasional contacts of anglers also indicated that the brook trout were being caught quite easily. As a means of checking the extent of repopulation of the stream by survivors or migrants, and to determine the survival of the planted brook trout during a 1-year period, the stream was again treated with rotenone in October 1958 and a careful search was made for all the fish killed during the treatment. The table summarizes the results of these two treatments with rotenone.

Survival of Stocked Trout

After the first application of rotenone, 858 4- to 8-inch brook trout were planted in Six Mile Run on September 6, 1957. On October 7, 1957, an additional 3,000 3- to 6-inch brook trout were stocked in the stream. These two plantings were not marked because presumably they were the only brook trout present in the stream at that time. The following spring, before the fishing season opened, two plantings of fin-clipped trout were made: 800 6- to 8-inch brook trout on March 11, and 100 7- to 9-inch rainbow trout on March 15, 1958.

On the day before the marked trout were planted, a fish barrier and trap were placed in Six Mile Run near its mouth. Thirteen brook trout were taken in the downstream trap on March 11, the day of the planting; but although the trap was checked daily thereafter until March 29, 1958, no additional trout were observed moving downstream.

Of the 800 marked brook trout and 100 marked rainbow trout that were stocked in March, just prior to the fishing season, only 1 rainbow and no brook trout were recovered when the stream was treated in October. Many of these trout were taken by anglers during the season, but no detailed creel census was made. Of the 3,858 fingerling brook trout planted in

SPECIES COMPOSITION OF FISH COLLECTED WITH ROTENONE IN SIX MILE RUN, WARREN COUNTY, PENNSYLVANIA

Species	Fish collected in September 1957		Fish collected in October 1958			
	Number	Percent of total number	Number	Percent of total number	Weight in pounds	Percent of total weight
Ichthyomyzon sp.	1	----------	5	----------	0.04	----------
Salmo trutta	325	26.3	210	7.0	8.99	8.2
Salmo gairdneri	1	----------	1	----------	.26	----------
Salvelinus fontinalis	5	----------	83	2.8	7.35	6.7
Catostomus commersoni	69	5.6	318	10.7	33.70	30.7
Hypentelium nigricans	38	3.1	168	5.6	29.56	26.9
Moxostoma sp.	------	----------	1	----------	.34	----------
Gila elongata	10	----------	20	----------	.13	----------
Semotilus atromaculatus	81	6.6	229	7.7	4.36	4.0
Semotilus margarita	39	3.2	30	----------	.31	----------
Hybopsis micropogon	------	----------	1	----------	.01	----------
Rhinichthys cataractae	16	----------	56	----------	.61	----------
Rhinichthys atratulus	452	36.6	612	20.5	3.72	3.4
Parexoglossum laurae	------	----------	2	----------	.03	----------
Notropis photogenis	------	----------	32	----------	.30	----------
Notropis rubellus	18	----------	8	----------	.04	----------
Notropis cornutus	12	----------	38	----------	.45	----------
Pimephales notatus	------	----------	16	----------	.16	----------
Campostoma anomalum	1	----------	762	25.5	17.51	16.0
Percina maculata	------	----------	26	----------	.22	----------
Etheostoma nigrum	14	----------	71	2.4	.19	----------
Etheostoma blennioides	------	----------	25	----------	.24	----------
Etheostoma caeruleum	------	----------	71	2.4	.28	----------
Etheostoma flabellare	23	----------	184	6.2	.72	----------
Cottus bairdi	131	10.6	14	----------	.18	----------
Total	1,236	92.0	2,983	90.8	109.70	95.9

the fall of 1957, only 83 (or 2.2 percent) were present in October 1958. Again, many of these were large enough to be taken by anglers during the fishing season. Despite the fact that no brown trout were planted after the treatment with rotenone in 1957, 210 brown trout were collected in October 1958. Some of these fish were mature (10 to 13 inches in length), but the majority were young-of-the-year fish, presumably the result of spawning which occurred after the initial treatment with rotenone.

Discussion

The use of rotenone for collecting samples of fish has been discouraged for fear of depressing the fish population in a stream for a long period. Our experience on Six Mile Run would indicate that although the use of rotenone appears to be an excellent procedure for collecting a large number and variety of fishes, rapid repopulation of large sections of stream takes place within a short time. In spite of our attempts to eradicate the

brown trout and restock brook trout only, the second treatment indicated that brown trout were still the dominant game fish in the stream. Elimination of fish populations may be possible with the use of rotenone, but only when special precautions are taken to treat the stream in a very thorough manner such as was outlined by Lennon and Parker (1959).

Sixteen species of fish were identified as being present at the time of first treatment (see table), when more than 90 percent of the population consisted of brown trout, white sucker, hog sucker, creek chub, pearl dace, blacknose dace, and mottled sculpin. This evaluation is based on a large representative sample of the fish preserved and field notes of the fish which were discarded.

During the second treatment 1 year later, 23 species of fish were collected, including all of the 16 species taken the previous fall. Because of their small size and scarcity in the population, some of these additional species could have been overlooked in the first treatment. Three minor changes in the fish population were noted during this 1-year period, but these had little effect in altering the species composition of the population. Brook trout were more numerous during the second treatment; many of these were survivors from the fingerling stocking. Stoneroller minnows greatly increased in abundance, and the mottled sculpin decreased. However, the fish population at the time of the second treatment still consisted mainly of brown trout, white sucker, hog sucker, creek chub, and blacknose dace.

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A collapsible rubberized-fabric dam of novel design has recently been invented by Norman M. Imbertson of the Los Angeles Department of Water and Power for use in areas where drainage and flow regulation are problems. Although its applicability to fish-pond construction has not been tested, there apparently are good possibilities for its adaptation to use in fish husbandry.

Mr. Imbertson described the development and application of his invention in a paper entitled "The Automatic Collapsible Membrane Type Rubber Dam and Its Uses," presented at the American Society of Civil Engineers Science Convention, Reno, Nevada, in June 1960.

DIRECT TRANSFER OF RAINBOW TROUT SWIM-UP FRY TO PRODUCTION RACEWAYS

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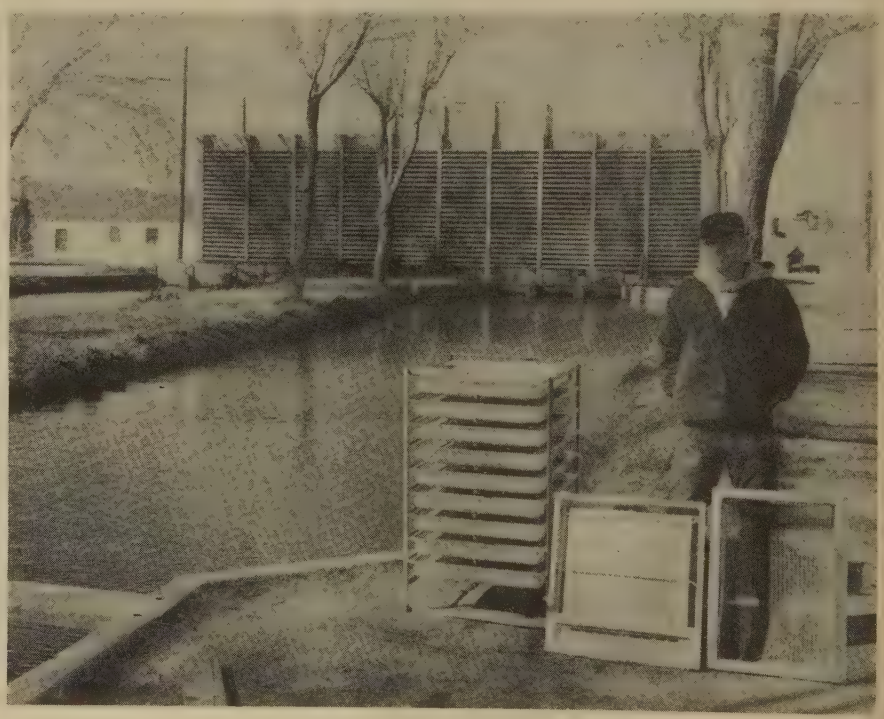
THE ADVENT OF A PRACTICAL INCUBATOR FOR TROUT EGGS (Burrows and Palmer 1955) suggested that it might be possible to dispense with troughs and nursery tanks in routine trout production. Accordingly, the California Department of Fish and Game made tests to explore this possibility. The results have been summarized in the table.



A raceway pond at the Mojave River Hatchery, of the type used in these experiments. The aerator in the background dissipates gases from the well water supply. The type of incubator used is shown in the foreground.

When rainbow trout fry were swimming up well and ready to feed, they were transferred to ponds. The fry were put into the head pond of a raceway series of the type appearing in the photograph. (These ponds are 100 feet long and 10 to 12 feet wide at the bottom, and have sloping clay sides and gravel bottoms. Depth is adjustable up to 3 feet.) The flow through the pond was reduced from the usual 3 cubic feet per second to 1/3 cubic foot per second in tests 1 and 3, and to 1 cubic foot per second in tests 2 and 4, in order to create more favorable conditions for the tiny fry. Ponds were not cleaned until the trout reached a size of 30 per ounce or larger. Water levels were maintained at approximately 2 feet.

The first test was conducted at Moccasin Creek Hatchery. The fish were given dry feed in crumble form. Initially

SURVIVAL OF TROUT FRY PLACED IN RACEWAY PONDS AT SWIM-UP STAGE

	Test 1 Moccasin Creek Hatchery	Test 2 Mojave River Hatchery	Test 3 Moccasin Creek Hatchery	Test 4 Mojave River Hatchery
Date fry were placed in raceways	5-21-58	12- 3-58	5-12-59	5-11-59
Termination date	8-19-58	3-27-59	7-17-59	1- 4-60
Length of test--days	90	114	66	238
Number of fry placed in raceways	216,700	254,000	247,700	300,000
Brood-stock strain	Mt. Whitney spring-spawning	Hot Creek fall-spawning	Mt. Whitney spring-spawning	Mt. Whitney spring-spawning
Number of survivors	53,010	154,000	157,568	1/ 250,000
Percentage of survival	24.5	60.6	63.6	2/ 83.3
Size	3/ 7.8	3/ 3	3/ 18.9	4/ 8

1/ Inventory was taken during five grading operations made between October 9, 1959, and January 4, 1960. The figure given represents the total of several partial counts adjusted to represent status of entire group on January 4, 1960.

2/ From estimates. 3/ Per ounce. 4/ Per pound.

the food was broadcast by hand about every 2 hours between 8:00 a.m. and 5:00 p.m. Later the feeding technique was changed to include use of a dispenser consisting of a 2-pound coffee can, with a 1/2- by 2-inch slot cut in its side near the bottom, attached to a 10-foot wooden handle. With this dispenser the dry feed could be placed very near the surface of the water, to reduce the chances of the food's sinking rapidly. This appeared to create better utilization of the food. This dispenser was illustrated and described by Leitritz (1959: 95). The discussion of test 3 includes additional details about feeding.

Several factors contributed to the low survival (24 percent) in this first test. Such factors included a disease outbreak diagnosed as a nutritional disorder (although fish eating the same sort of food in troughs did not suffer increased losses), predation by Brewer blackbirds, and unusual difficulties with screens and water flows, aggravated by the small size of the fish.

The reduced flow encouraged prolific algal growth, which continually plugged the fine screens and occasionally caused fish loss when the water overflowed them. However, by the time the fry were large enough to move actively around the pond, the algal growth had disappeared--for no clear reason--in both tests at Moccasin Creek Hatchery. The fish were never seen to feed on algae, and no changes were made in water flow or screen sizes.

The second test was conducted at the Mojave River Hatchery, located about 100 miles east of Los Angeles. The fry were started on No. 1 dry feed, which was fed by means of a feed dispenser as at Moccasin Creek Hatchery.

At hourly intervals the fish were fed amounts calculated from standard feeding charts based on body weight and water temperature. The amount used during the first few days was about double the recommended amount, in order to "reach" all of the fish on each of the frequent feedings. As the fish grew, the frequency of the feedings was reduced. Dry feeds of various brands were used throughout the experiment, and standard feeding charts were followed closely.

Test 2 gave 60 percent survival after 114 days, with excellent growth. There were two principal causes of mortality. The first was cannibalism, which was observed frequently when the fish were

small. Early in this experiment, it was seen that many of the fish were taking little, if any, feed. By the fifth week, an extreme variation in size existed and cannibalism became noticeable. Within a few minutes of observation, two or three larger fish could be seen with tails protruding from their mouths. This continued for several days, until few small fish remained. Major losses also resulted from the plugging of the screens by algae, which raised the pond level as much as 9 inches. Many fry were stranded among the weeds and roots along the bank as the water level dropped rapidly when screens were cleaned.

Survival was 60 percent in test 3, the second test at Moccasin Creek Hatchery; whereas a normal survival in troughs at Moccasin Creek was 85 to 90 percent from alevin stage to comparable size. The fish in tests 1 and 3 were fed starter crumbles at first. In 2 weeks, they were gradually changed to No. 1 crumbles. The changeover required 5 days to complete; that is, one feeding of No. 1 crumbles was given on the first day of changeover, two feedings of No. 1 crumbles were given on the second day, etc., until the fish were receiving 100 percent No. 1 crumbles on the fifth day. The fish were fed five times a day--at 8:00 a.m., 11:00 a.m., 1:00 p.m., 3:00 p.m., and at 5:00 p.m. In test 1, the fish were changed briefly to a 100-percent diet of beef liver when evidence of a nutritional disorder appeared and mortality increased. Within a week, the mortality dropped to normal and the symptoms of suspected malnutrition disappeared. The fish were then returned to the 100-percent dry diet with no further trouble.

A fourth experiment with about 300,000 fry, the second test made at Mojave River Hatchery, was begun May 11, 1959. This time a special effort was made to get all of the fish to start feeding. The same type of feed and the same methods were used. However, as nearly as possible, fish were fed at half-hour intervals for the first few days. This resulted in more uniform initial growth and reduced subsequent loss by cannibalism. The importance of frequent feeding cannot be overstressed in starting fry on dry food. Black polyethylene film, 0.004 inch thick, placed on the sides of the pond, kept the small fish from being stranded when the water level dropped. Fish were graded into two sizes after 70 days in the pond, with excellent results.

This fourth test was an outstanding success, with high survival and excellent growth of good-quality fish. The possibility of an error in eyed-egg count and the lack of a reliable inventory of resulting fish made it impossible to calculate survival accurately. It was estimated to have been about 83 percent.

Fry used in tests 1 and 3 were hatched in baskets in troughs, in the absence of an incubator at Moccasin Creek Hatchery. Fry used at Mojave River Hatchery were hatched in a Roberson incubator (Leitritz 1959: 57).

In all four tests, the quality of the pond-reared fish compared favorably with that of fish from the same eggs reared in the usual way. Generally speaking, personnel at the two hatcheries consider the test fish equal or superior to regular production fish.

To summarize, survival approaching that ordinarily obtained with troughs was achieved when fry were transferred directly from incubators to raceways, with appropriately modified rearing methods. The growth and quality of fish were both excellent.

If the subsequent experience confirms these preliminary tests, there will be several important implications. Intermediate facilities, such as troughs and nursery tanks, can be eliminated from some trout production operations, thereby reducing capital outlay and maintenance costs. Moreover, large rearing-pond installations, designed primarily for production of catchable-sized trout, will be able to increase production of fingerlings greatly without adding expensive troughs or tanks, by substituting fingerlings for catchables or by using ponds when they are not needed for catchables.

These preliminary tests have already increased the versatility of Mojave River Hatchery, which lacks troughs or nursery

tanks. Previously it had been considered necessary to import from other stations fingerlings at a size of 60 to 125 per ounce to stock in the raceways of the Mojave River Hatchery. Now, however, this station can import eyed eggs rather than fingerlings when that is advantageous.

Acknowledgments

The participation of L. E. Nixon and D. L. Evins in these experiments is gratefully acknowledged. Earl Leitritz and Alex Calhoun encouraged the project and assisted with the final report.

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RESEARCH ON FUNGI ASSOCIATED WITH DISEASES OF FISH AND FISH EGGS

A formal report on the program of research on fungi pathogenic or parasitic to fish (THE PROGRESSIVE FISH-CULTURIST 21 (3): 120) will be made in the near future.

To date, 125 unifungal isolates have been obtained from 57 infected specimens received in our laboratory. Specimens have been received from 14 States and include 28 different host species representing 18 genera. Lists of host species and locations will be supplied upon request.

Isolates of species representing the following genera have been found associated with diseased fish or fish eggs: Saprolegnia, Achlya, Isoachlya, Aphanomyces, Pythium, Allomyces, Leptomitus, and Apodachlya. In addition, species representing at least four genera of the Fungi Imperfecti have been isolated. Twelve isolates from material received failed to grow under cultural conditions.

Studies on the identification of species are in progress. To date, the following species have been identified: Saprolegnia parasitica (8 isolates), S. diclina (7 isolates), S. ferax (5 isolates), S. delica (4 isolates), Saprolegnia spp. (2 well-defined isolates, probably representing a new species), Aphanomyces laevis (2 isolates), Leptomitus lacteus (2 isolates), and Allomyces anomalis (1 isolate). Specific identifications within the other genera mentioned are in progress.

It is hoped that the scope of this project will be expanded in the near future. The undertaking is currently supported by the Virginia Agricultural Experiment Station. Studies on the host-parasite relations are now underway, and studies on the control of these fungi are contemplated. We have recently completed the installation of an air-conditioned transfer room with adequate refrigerated storage space. It is now possible for us to handle greater numbers of specimens and to isolate without danger of contamination.

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As it is important that this research program include studies on fungal infections in all parts of the country, fishery biologists and hatchery managers are urged again to notify Dr. Scott (by postal card) of the availability of material--fungi from dead and live fish, fungus-infected fish and eggs, and fungus cultures. Dr. Scott will supply containers and full information about handling and shipping the material.

---THE EDITOR.

IMMERSION STAINING AS A METHOD OF MARKING SMALL SALMON

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IN RECENT YEARS fishery biologists of the Bureau of Commercial Fisheries in Alaska have fin-clipped large numbers of pink and chum salmon fry (Oncorhynchus gorbuscha and O. keta) which were used in mark-and-recover experiments of 1 to 3 days' duration. During these experiments it became apparent that the conventional fin-clipping method of marking had several disadvantages, including high cost in terms of labor to mark the fish and some loss of fish from handling. To eliminate these problems, other methods of temporarily marking fry were sought, and staining by immersion in a dye solution seemed to be one of the most promising possibilities.

Starfish have been successfully stained by immersion in dye solutions. Loosanoff (1937) marked the Atlantic coast starfishes, Asterias forbesi and A. vulgaris, with Nile Blue Sulphate. Vernon (1937) obtained encouraging results with Neutral Red in staining Asterias. More recently, Feder (1955) used a dye similar to Nile Blue Sulphate and also Neutral Red to stain the Pacific coast starfish, Pisaster ochraceus.

First attempts in Alaska at immersing fry in a staining solution were made in 1956 and 1957, and more extensive trials with various dyes were conducted in 1958. Experiments utilizing a weak solution of Neutral Red produced the best results, and during 1958 and 1959 approximately 170,000 pink and chum salmon fry were successfully stained for use in marking experiments.

Methods

Various dyes were diluted with fresh water from streams where experiments were conducted. Dyes tested included Neutral Red and Alizarin Red, Malachite Green, Eosine Yellowish, Nile Blue A, and Toluylene Blue.

Concentrations ranged from 1:50,000 to 1:1,000,000, and periods of immersion from 4 to 20 hours. Most experiments were made with pink, chum, and coho (O. kisutch) salmon fry from 1 to 1.5 inches long. A few experiments were conducted on coho and red (O. nerka) salmon fingerlings from 2.5 to 5 inches long.

An ordinary galvanized washtub was used to hold the fish while they were in the stain solution (figure 1). When anoxemia occurred, as evidenced by fish turning on their sides and gasping, aeration was supplied by dipping and pouring the solution or by using a new solution. Aeration was usually necessary when water temperatures exceeded 50° F.

Experimental Results

To determine the staining technique most satisfactory from the standpoint of maximum color retention and minimum mortality, tests were made with pink and chum salmon fry immersed in various concentrations of Alizarin Red, Malachite Green, and Neutral Red. In these tests, water temperatures ranged between 33° and 36° F. Most satisfactory results in

FIGURE 1.--Equipment used in staining large numbers of salmon fry by immersion. Note the rectangular fry traps, which are used for sampling migrating fry.

these experiments with pink and chum salmon fry were obtained with Neutral Red solutions at concentrations of 1:95,000 and 1:270,000 (table 1).

Chum salmon fry seemed to be much hardier than pink fry, as evidenced by only a 20-percent mortality of chum fry, compared with a 90-percent mortality of pink fry, 7 days after staining. Cumulative deaths of unstained chum fry at the end of 7 days further demonstrated the greater hardiness of these fry. Chum fry also differed from pink fry in color retention. Stained chum fry faded about twice as rapidly as did pink fry, but both were easily detected among unstained fry 3 days after staining.

Additional staining experiments were made with pink and chum fry, using Eosine Yellowish, Nile Blue A, and Toluylene Blue in concentrations ranging from 1:90,000 to 1:450,000 for immersion periods of 4 to 20 hours. Limited success was obtained only with Nile Blue A (1:300,000 concentration, 7 hours), which produced light blue tinges on the head and silvery surfaces of the body. Identification of stained fry was difficult 2 hours later.

Coloration first became apparent on the mandibles, then on the silvery portions of the lateral and ventral surfaces of the body, and finally on the fins and the pigmented portions of the body. Fading of color from the body parts was in the reverse order. Coho fry, the most highly colored of the salmon tested, required the longest period of immersion and were the most difficult to detect among unstained fish.

In one experiment (figure 2), fin-clipped coho fry in lots of 25 were subjected to Neutral Red dye in five concentrations ranging from 1:25,000 to 1:500,000 for immersion periods ranging from 2 to 6 hours. The fish were clipped so that the lots could be distinguished from each other in the common live box. Satisfactory results were obtained by using a concentration of 1:250,000 for 6 hours; concentrations of 1:100,000 and stronger were too toxic, and a concentration of 1:500,000 was too weak for satisfactory staining. Mortality increased with the higher concentrations. The unstained control lot suffered the lowest number of deaths, indicating that dye was somewhat harmful even at the lowest concentrations.

Further testing included an experiment in which 175 red salmon smolts were successfully stained in a Neutral Red solution at a concentration of 1:300,000 for

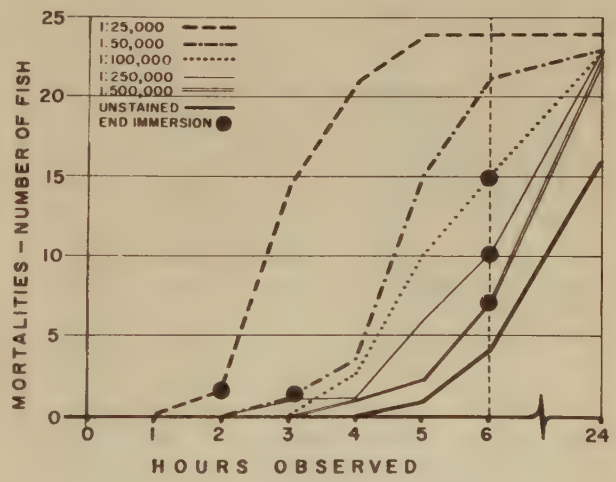

FIGURE 2.--Mortality of 25-fish lots of fin-clipped coho salmon fry subjected to various concentrations and immersion periods in Neutral Red dye solutions.

TABLE 1.--Effects of dyes at different concentrations on pink and chum salmon fry

Dye and concentration	Number stained: Pink	Number stained: Chum	Number unstained (control): Pink	Number unstained (control): Chum	Hours of immersion	Days after immersion	Effects	Cumulative deaths: Pink: Stained	Cumulative deaths: Pink: Unstained	Cumulative deaths: Chum: Stained	Cumulative deaths: Chum: Unstained
Alizarin Red:											
1:1,000,000	5	5	0	0	4	0.0	No color, no effects.	0	-------	0	-------
1: 270,000	5	5	0	0	7	.0	Slight color, faded after 2 hours.	0	-------	0	-------
1: 180,000	5	5	0	0	6	.0	Light pink, faded after 4 hours.	0	-------	0	-------
1: 50,000	5	5	0	0	7	.5	Fins pink, faded after 6 hours. Only 2 normal fry remained.	1/ 6	-------	(1/)	-------
Malachite Green:											
1:1,000,000	5	5	0	0	4	0.0	Fins light green; acute distress.	0	------	0	-------
1: 270,000	5	5	0	0	6	0	Fins green; lethal.	5	-------	5	-------
Neutral Red:											
1:1,000,000	5	5	0	0	4	0.0	Fins pink; faded after 4 hours.	0	-------	0	-------
1: 270,000	5	5	0	0	6	1.0	Fins and head bones medium red; ventral body surface pink; color retained 24 hours.	0	-------	0	-------
1: 90,000	5	5	0	0	6	1.0	Fins and body deep red; lethal.	5	-------	5	-------
1: 95,000	96	100	98	100	5	.2	Deep red; heavy pink mortality.	17	0	-------	-------

(Continued)

TABLE 1.--Effects of dyes at different concentrations on pink and chum salmon fry--Continued

Dye and concentration	Number stained		Number unstained (control)		Hours of immersion	Days after immersion	Effects	Cumulative deaths			
								Pink		Chum	
	Pink	Chum	Pink	Chum				Stained	Unstained	Stained	Unstained
Neutral Red (continued):											
1: 95,000	96	100	98	100	5	1.0	Pinks deep red; chums faded on belly.	53	3	4	0
1: 95,000	96	100	98	100	5	2.0	Pinks faded on belly; chums faded overall, less than half original color remaining.	74	4	13	0
1: 95,000	96	100	98	100	5	3.0	Pinks faded but still readily identified; chums lost most of original color.	83	7	17	0
1: 95,000	96	100	98	100	5	4.0	First loss of color on ventral heads of pinks; chums with only pink tinge.	86	7	18	0
1: 95,000	96	100	98	100	5	5.0	Pinks faded but color still evident.	86	9	19	0
1: 95,000	96	100	98	100	5	6.0	Pinks lost over half original color; chum color no longer evident.	88	10	20	0
1: 95,000	96	100	98	100	5	7.0	Pink color nearly gone, but still evident.	90	11	20	0

[1] Total of 6 mortalities, pink and chum combined.

7 hours. The smolts were well colored and suffered no ill effects.

Standardized Procedure

Although we found that Neutral Red used at a concentration of 1:270,000 for 6 hours produced very satisfactory results in terms of coloration and lack of mortality (table 1), we conducted an additional experiment using Neutral Red at a concentration of 1:300,000 for 7 hours (table 2). No unstained controls were used, so the mortality from holding and staining cannot be separated.[1/] This is not too important, however, considering the low total mortality for all three species. This experiment demonstrated that low mortality and satisfactory color retention for several days could be attained with Neutral Red at a concentration of 1:300,000 with 7 hours' immersion. Accordingly, all further staining was done by this method, and it was designated "standardized procedure."

Under field conditions the approximate concentration was obtained by pouring 12 gallons of stream water into a washtub, adding 0.15 gram of Neutral Red, and dipping and pouring portions of the solution back into the tub until the dye was thoroughly dissolved.

Staining Versus Fin-Clipping

A series of eight experiments was conducted to compare the mortality rates of fin-clipped and the stained pink salmon fry, and to compare recovery rates of fry marked by the two methods. Groups of 650 to 1,050 fry were used, the groups compared in each experiment being of almost equal size. In each experiment one lot of fry was marked with a dorsal fin-clip, and one lot was stained with the standard Neutral Red solution. To recover from the shock of marking and handling, the fry were held for 3 hours before being released. The percentage of mortality at the end of the 3-hour holding period is shown in figure 3.

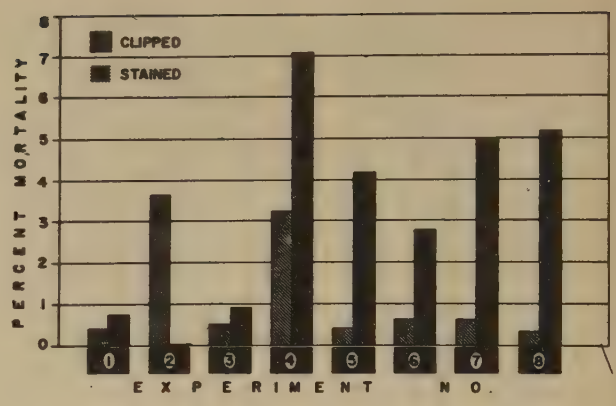

FIGURE 3.--Mortality rates of eight experimental groups of pink salmon fry 3 hours after fin-clipping and immersion staining. Groups contained between 650 and 1,050 fish.

At the end of the 3 hours, the fry were placed in a single container where complete mixing occurred. They were released at night at a point several hundred yards upstream from a series of rectangular traps and were recovered in the traps within an hour. Thus, the higher mortality rate of the clipped fry (figure 3) could not significantly influence the recovery rate. The percentages of recovery of the fin-clipped and the stained fry were similar, indicating that fry marked by either method are equally likely to be recaptured (figure 4).

For marking salmon fry, staining was found to be much less tedious and time-consuming than fin-clipping. After being given simple instructions, untrained personnel could quickly master the technique. To attain proficiency in fin-clipping, however, considerable training and experience are required. Even more important, mortality among the fish as a result of staining is less than the mortality that occurs with fin-clipping. Because of the difficulty of fin-clipping small salmon, all of the fry used in our experiments were anesthetized before they were clipped.

Stained fry in a mixture of captive stained and unstained fish can be readily

[1/] In another experiment, losses were 10 percent among control pink and 5 percent among control chum salmon fry held 7 days.

TABLE 2.--Effects of immersing salmon fry and fingerlings in Neutral Red Stain at a concentration of 1:300,000 for 7 hours

Days after staining	Color retention			Cumulative deaths					
	Pink fry (120 in sample)	Chum fry (276 in sample)	Coho fingerlings (7 in sample)	Pink		Chum fry		Coho fingerlings	
				Number	Percent	Number	Percent	Number	Percent
1	Excellent color.	Excellent color.	Good color.	2	1.7	0	0.0	0	0.0
2	Excellent color, no fading.	Excellent color, slight fading.	Good color, slight fading.	9	7.5	4	1.4	1	14.3
3	Good color, some fading.	Fair color, some fading.	Fair color, some fading.	9	7.5	4	1.4	1	14.3
4	Fair color, continued fading.	Poor color, continued fading.	Poor color, continued fading.	11	9.2	5	1.8	1	14.3
5	Fair color on ventral surfaces.	Poor color, tinge only.	Very poor color.	15	12.5	15	5.4	1	14.3
6	Poor color.	Poor color.	Very poor color.	15	12.5	18	6.5	1	14.3
7	Poor color but still evident.	Color not evident.	Color not evident.	18	15.0	26	9.4	1	14.3

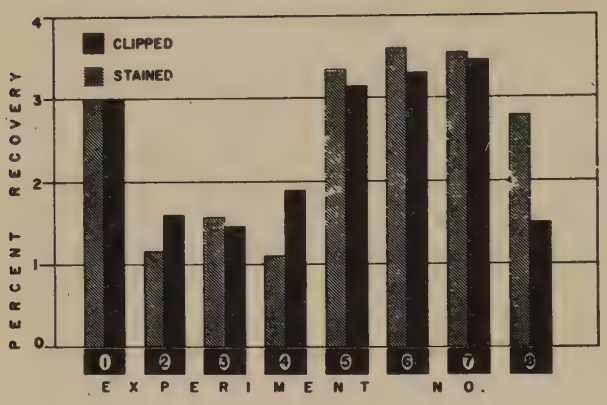

FIGURE 4.--Recovery rates of eight experimental groups of fin-clipped and stained pink salmon fry used to test recapture of fish marked by two methods. Groups contained between 650 and 1,050 fish.

detected by merely glancing at the group, in contrast to the careful examination of individual fish that is required to separate fin-clipped fish. Detection of stained fry was satisfactory for up to 7 days for pink salmon and up to 72 hours for the other species tested.

The staining technique is superior in all respects to fin-clipping for short-term experiments in which detection of marked fish is necessary for only a few days. Although only small salmon have been tested in our experiments, the Neutral Red staining technique would probably be adaptable to any light-colored small fish.

Summary

1. An aqueous Neutral Red solution at a concentration of 1:300,000 and an immersion period of 7 hours yielded the best results in staining small salmon.

2. Concentrations which were greater than 1:100,000 or less than 1:500,000 were unsatisfactory, the former being toxic and the latter failing to stain the fish adequately.

3. The Neutral Red solution produced a light to medium red color on juvenile pink, chum, coho, and red salmon.

4. Color retention was satisfactory for 72 hours when an aqueous Neutral Red solution at a concentration of 1:300,000 and an immersion period of 7 hours were used. Coho fry were the most difficult to identify because of natural pigmentation. Pink fry retained visible coloration the longest of any of the species tested.

5. Mortality during a 7-day holding period was 15 percent for pink, 9 percent for chum, and 14 percent for coho salmon in one experiment using Neutral Red at a concentration of 1:300,000 and a 7-hour immersion period. Losses resulting from holding and staining were not separated.

6. Among pink salmon fry, immediate mortality attributed to staining was generally much less than that attributed to fin-clipping.

7. Recovery rates of stained and fin-clipped pink salmon fry were quite similar in mark-and-recover experiments.

8. Approximately 170,000 stained pink and chum salmon fry were used in short-term mark-and-recover experiments in Alaska during 1958 and 1959.

9. Experiments with Alizarin Red, Eosine Yellowish, Malachite Green, Nile Blue A, and Toluylene Blue did not produce satisfactory results.

Acknowledgments

The authors wish to express their appreciation to Richard T. Myren, whose preliminary fry-staining experiments in 1956 and 1957 formed the basis for their successful technique; and to James Kirkwood and Richard Hobart, who conducted some of the experiments described here.

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A STAINING METHOD FOR MARKING LARGE NUMBERS OF SMALL FISH

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MARKING FISH for later recognition has long been a practice of fishery biologists desiring to obtain data for estimates of abundance, to study extent of movements, and for other special purposes. This paper describes a method (staining) that is useful for marking large numbers of small fish for short-term studies in which recognition of individuals is unimportant.

In preliminary tests, live fish (mostly fathead minnows, Pimephales promelas) were totally immersed for varying periods in varying dilutions of the following stains:

Acriflavine	Para Rosanilin (Hydrochloride)
Anilin Blue	Phenosafranine
Bismark Brown Y	Ponceau S X
No. 517 Erythrosine	Proflavine
Fast Green	Rhodamine B
Fluorescein	Rhodamine 6 G
Janus Green	Rosanilin Hydrochloride
Magdala Red	Safranin Bluish
Mercurochrome	Safranin O
Methylene Blue	Substitute Phenosafranine
Para Rosanilin (Base)	Trypan Blue

Bismark Brown Y gave the best results and subsequently was used successfully on the following species of fish:

Carpiodes carpio	Pimephales promelas
Moxostoma erythrurum	P. notatus
Semotilus atromaculatus	Phenacobius mirabilis
Notropis lutrensis	Campostoma anomalum
N. umbratilis	Chrosomus erythrogaster
N. stramineus	Ictalurus melas
Ictalurus natalis	Etheostoma spectabile
I. punctatus	Micropterus punctulatus
Pylodictis olivaris	Lepomis cyanellus
Noturus flavus	L. megalotis
Percina caprodes	
P. phoxocephala	

When fish were kept in the dye for 3 hours at a concentration of 1:5,000 at 65° F., or 1:20,000 at 85° F., they became brightly colored and remained easily recognizable for 1 to 2 weeks.

In reporting on tests of several biological stains used for marking fish by injection, Dunn and Coker (1951) wrote that Bismark Brown Y caused death within 30 hours. In my experiments, dyed fish that were kept in the laboratory retained recognizable color for as long as 2 weeks and appeared to be as vigorous at the end of the test period as at the beginning. Cross sections of fish that were sacrificed immediately after their removal from the staining solution indicated that the dye was concentrated near the surface of the body. In fish sacrificed 1 day later, the dye was partly dispersed through the body musculature and showed a slight concentration near the surface and in the kidneys.

In field studies of populations and of dispersion of small fishes in streams, Bismark Brown Y was used to mark as many as 1,500 fish (mostly minnows) simultaneously. The fish were held in a tank containing 150 gallons of the dye solution, which was aerated by means of a circulating pump. The 3-hour exposure

to Bismark Brown Y weakened the fish; and they were placed in a live box in the stream until they recovered, at which time they were released into the stream. The period of recovery was approximately 10 minutes.

Acknowledgments

The work on this paper was done as a part of a larger study on two Kansas streams, made possible by support of the Kansas Forestry, Fish and Game Commission. I wish to thank Dr. Frank B. Cross for helpful suggestions.

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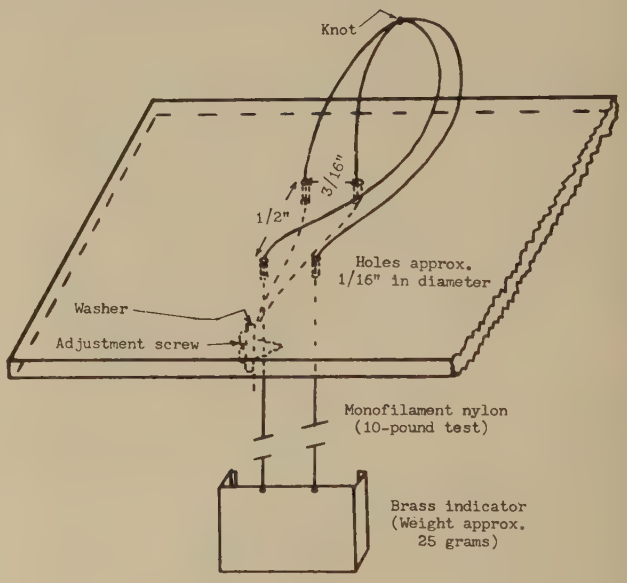
AN APPARATUS FOR MEASURING THE GIRTH OF FISH

Recently biologists have been using length-girth data in studies on gill-net selectivity. With girth-measuring techniques already described, some error has been likely to occur because of the softness of the fish belly in the region of maximum girth and because of variations in the tension of the measuring tape or cord. This apparatus has been designed to measure girths of large numbers of fish quickly and accurately.

A section of plexiglass is set into the top of a table (see photograph). A scale is fixed to a wood support suspended by a hinge to the underside of the table and held in a vertical position by two cords. Two strands of monofilament nylon go through holes in the table (see drawing), forming a loop over the table and supporting a brass indicator that moves along the scale in accordance with the girth of the fish. The weight of the indicator maintains the tension of the loop on the fish body.



A bottle of known circumference is set in the loop and its value checked with the scale reading. If this does not equal the circumference of the bottle plus 0.1 inch (added because the bottle does not accommodate to the table surface), the nylon is moved up or down till the correct reading shows on the scale; then the adjustment screw is reset appropriately.

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THE USE OF SAPONIN TO CONTROL PREDACEOUS FISHES IN SHRIMP PONDS

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EXPERIMENTS IN POND CULTURE OF SHRIMP have received an increasing amount of interest from fishery biologists in the United States as the supply of shrimp from trawling operations has become insufficient to meet the existing demand, especially as there is considerable acreage of marshland on the Gulf coast that is available for shrimp farming (Anonymous 1958). Before the controlled culture of shrimp can be put on a commercial scale in the United States, a considerable amount of information on management of this type of pond is necessary. However, scientific investigations in this field are very meager. Even in Taiwan, where the Chinese fish farmers have developed the culture of shrimp in milkfish (Chanos chanos) ponds for centuries, the culture of shrimp alone is yet unpractical and unprofitable, though the price of shrimp there is usually approximately three to five times higher than that of milkfish. The main reason for this is probably the high mortality of shrimp, caused by a number of factors--including temperature, salinity, predation, and cannibalism--which the fish farmer has not yet been able to control.

In the United States, Johnson and Fielding (1956) reported successful spawning of the white shrimp (Penaeus setiferus) in a concrete tank as a preliminary step in the development of commercial shrimp culture in ponds. Lunz (1958) experimented with the pond cultivation of shrimp in South Carolina for more than 10 years, and concluded that the eating of shrimp by predatory fishes is possibly one of the important factors causing high mortality of shrimp. It has been noted also that in addition to active predatory species, the presence of herbivorous fishes such as mullet (Mugil) might conceivably influence the shrimp production, as they compete with shrimp for food and/or for living space. Consequently, the elimination of fish from shrimp rearing ponds should be considered as one of the major problems.

Saponin is a class name for a group of glucosides, extracted from a group of plants such as Sapindaceae, Sapotaceae, Camelliaceae, Leguminosae, Zygophyllaceae, Rhamnaceae, Rutaceae, Caryophyllaceae, and Scrophulariaceae. That this substance is poisonous to fish has appeared in earlier literature (Kobert 1904, Kofler 1927, Kofler and Schrutka 1925). In many parts of the world, people have used these plants as "fishing-plants" for poisoning the fish in rivers or in estuarine inlets of the sea from time immemorial. Use of tea-seed cakes for eradicating the undesirable species of fish in ponds before stocking is a traditional practice of Chinese fish farmers. This cake is the residue after expressing the oil from the seeds of Camellia, which contain 10 to 13 percent of saponin.

To determine the possibility of using saponin as a selective poison to control predaceous fishes in shrimp ponds, tests of the toxicity of this chemical to fish and to shrimps were made. The purpose was to locate its margin of safety between the

minimum disabling dosage for shrimp and the minimum strength necessary for fish control.

Toxicity of Saponin to Fishes and to Shrimps

To determine the toxicity of saponin to fishes and to shrimps in the laboratory, the bioassay methods given by Doudoroff (Doudoroff et al. 1951) were adopted. The source of water and the laboratory facilities used in this test were described in a previous paper by Tang and Chen (1959). Fish selected as test animals were the predaceous Elops saurus and the herbivorous Tilapia mossambica in the young stage (total length, 40 to 68 millimeters). The test shrimps were Caridina denticulata and Penaeus carinadus. The former is an inedible species of very small size and is widely distributed in the estuarine waters of shallow bays or inlets; whereas the latter species is raised on a commercial scale in association with milkfish in brackish-water ponds in very limited acreage on the Island. Young P. carinadus with total lengths ranging from 25 to 50 millimeters were used in this trial. All these test animals were collected from the canals of milkfish ponds in the Tainan Fish Culture Station. The saponin used in this experiment was extracted from the seeds of Camellia sinensis with alcohol. This colloidal extract was dried at a constant temperature of 60° C. and then powdered.

The results of this experiment showed that the lethal dosages of this chemical (for 100 percent of the test animals dead in 24-hour exposure) for the fingerlings of E. saurus and T. mossambica were, respectively, 1.0 and 1.5 p.p.m. at the temperature of 29±1.5° C. and salinity of 24.6 $^{0}/_{00}$. By comparative test conducted in a similar environment, the lethal dosages of this material for C. denticulata and P. carinadus were 50.0 and 75.0 p.p.m., respectively. The resistance of shrimps to the toxicant saponin, based on this bioassay, is approximately 50 times higher than that of fish. Apparently saponin is a most promising chemical for use in the control of predaceous fishes in shrimp ponds.

Practical Control

Control experiments in the field were made in three rectangular 0.25-hectare ponds equipped with concrete sluices. Before the start of this testing, these ponds were drained and refilled with water through a conical screen net made of ramie to keep out any small wild fish or shrimp. Young shrimps of a mixture of four main species -- P. penicillatus, P. bubulus, P. semisulcatus, and C. denticulata -- with total lengths ranging from 8 to 35 millimeters were stocked at the rate of approximately 5,000 to each of these ponds. These young shrimps had been caught with a triangular dip net in shallow bays or inlets where bunches of seaweeds had been planted to furnish suitable environment for the young shrimps to congregate. A week after the shrimps had been planted, 300 E. saurus and 150 Mugil cephalus with total lengths ranging from 40 to 290 millimeters were seined from the canals of milkfish ponds and immediately afterward were placed, in equal numbers, into each of these three ponds. Powdered saponin at the concentrations of 2.5, 5.0, and 10.0 p.p.m. was applied, respectively, to the three ponds 24 hours after the additional stocking with fish. At the time of treatment with saponin, the pond water was about 30 centimeters deep and had a temperature of 34° C. and a salinity of 24.6 $^{0}/_{00}$.

E. saurus began showing signs of distress within 15 minutes after the ponds were treated with the saponin. Both E. saurus and M. cephalus died within 6 hours after the treatment, except for some large individuals which were surfacing in the pond treated with 2.5 p.p.m. A check made by draining the ponds at the end of 24 hours showed that all the fish stocked in these three ponds were dead, but the shrimps were all alive and active.

A second field trial of saponin, using crumbled tea-seed cakes and following the same procedure as in the previous test, was conducted in the same ponds. The

concentrations of saponin were also 2.5, 5.0, and 10.0 p.p.m., respectively, in these three ponds and were arrived at by calculation of the percentage of saponin in the tea-seed cakes. The mean water temperature on the day of treatment was 26° C. The results of this trial showed that the saponin in the tea-seed cakes and the saponin extract were of equal effectiveness against fishes in shrimp ponds without causing apparent injury to the shrimp.

A third field test was conducted in the canals of milkfish ponds by use of crumbled tea-seed cake. Tea-seed cake in quantity sufficient to give a concentration of 5.0 p.p.m. saponin was used in the water of the canals in which various wild fishes and shrimps had gained entrance through the intakes covered with centimeter-mesh hardware screen. Shrimps (mostly C. denticulata) in cages were also placed in the canals for examination. The surface water temperature at the time of treatment with saponin was 28.5° C. This treatment killed various wild fishes--including E. saurus, Megalops cyprinoides, Chanos chanos, T. mossambica, Anguilla japonica, Mugil spp., Gobius spp., Dorosomatidae, Serranidae, and Engraulidae in various sizes--after a 12-hour period, but shrimps in both the canals and the cages were unaffected.

It is evident that in these three tests Camellia saponin in concentrations ranging from 2.5 to 10.0 p.p.m. and in the form of either powdered extract or seed cake per se successfully eliminated the undesirable fishes without injury to the shrimps. Further research on the effect of saponin treatment on the growth of shrimps and shrimp food organisms, however, is necessary before this material can be put to practical and extensive use in management of shrimp ponds.

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THE EFFECT OF TRICAINE METHANESULFONATE (M.S. 222) ON THE MOTILITY OF BROOK TROUT SPERM

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DURING SPAWN-TAKING in the fall of 1957, tricaine methanesulfonate (M.S. 222) was used to anesthetize brood-stock lake trout at the State fish hatchery at Marquette (Michigan) and brook and brown trout at the State fish hatchery at Grayling. An unusually high mortality of eggs ensued at Marquette, but there was a good hatch at Grayling. It was suggested that this difference might have been the result of slight differences in techniques used at the two hatcheries, and that the mortality at Marquette might have been caused by the anesthetic. A concentration of 2 grams of M.S. 222 to 7 gallons of water was used at Grayling, and 4 grams to 7 gallons at Marquette; though the anesthetized fish were rinsed in fresh water at both stations to remove all traces of chemical before the spawn-taking, gloves were used to handle the fish at Marquette, but not at Grayling. Water dripped from the gloves into the spawn-collecting pan, presumably carrying with it a small amount of M.S. 222.

Tests were made at Marquette to learn what effects various dilutions of M.S. 222 might have on brook trout sperm. Though the spawning season was nearly over, some males in the ponds were still ripe. Microscopic examination of the milt revealed a considerable variability in motility of the spermatozoa among different males. Although spermatozoa from some fish were completely immotile, five males produced sufficient quantities of motile cells for testing.

Procedure

A stock solution of M.S. 222 (75.5 p.p.m.) was made by dissolving 2 grams of the chemical in 7 gallons of water. For each test, stock solution was diluted with river water (which supplies the hatchery) to give 400 cc. of solution of a specified concentration of M.S. 222. Each test was made in a 600-cc. glass beaker. Several drops of milt were expressed from a fish into the solution, and the contents stirred immediately; the time was checked on an electric clock with a sweep second hand. To check motility, a drop of the mixture of milt and solution was put on a glass slide, covered with a cover glass, and examined under low power of a compound microscope. The first check was made 10 seconds after the milt was put in the solution, the second was made 20 seconds later, and subsequent checks were made at 30-second intervals until no further motility was observed. Fresh samples were taken from the mixture for each examination. Spermatozoa from all fish were checked in river water to be sure they were motile. Checks in river water were made 3 times during the 11 tests with the fourth male because it was handled for a much longer time than the other specimens.

A brief summary of this study was published by Sandoz Pharmaceuticals, Sandoz, Inc., Hanover, New Jersey, in an undated technical bulletin, "M.S. 222-Sandoz. The anesthetic of choice in work with cold-blooded animals."

DURATION OF MOTILITY OF BROOK TROUT SPERMATOZOA IN FRESH WATER, URETHANE, AND M.S. 222, ON DECEMBER 11, 1957

[+ = sperm motile; 0 = sperm immotile]

Test number	Male number	Solution[1]	Time (seconds) 10	30	60	90	120	150	180	210	240
1	1	FW--------	+	+	+	+	+	0	---	---	---
2	2	FW--------	+	+	+	+	+	0	---	---	---
3	3	FW--------	+	+	+	+	+	+	+	+	0
4	3	MS (75.5)	0	0	---	---	---	---	---	---	---
5	3	UR (2,995)	0	0	---	---	---	---	---	---	---
6	4	MS (75.5)	0	0	---	---	---	---	---	---	---
7	4	UR (2,995)	0	0	---	---	---	---	---	---	---
8	4	FW--------	+	+	+	+	+	0	---	---	---
9	4	MS (37.7)	0	0	---	---	---	---	---	---	---
10	4	MS (18.9)	0	0	---	---	---	---	---	---	---
11	4	FW--------	+	+	+	+	+	+	0	---	---
12	4	MS (9.4)	+	0	0	---	---	---	---	---	---
13	4	MS (9.4)	+	+	0	0	---	---	---	---	---
14	4	MS (4.7)	0	0	---	---	---	---	---	---	---
15	4	MS (4.7)	0	0	---	---	---	---	---	---	---
16	4	FW--------	+	+	+	+	0	---	---	---	---
17	5	FW--------	+	+	+	+	+	0	---	---	---
18	5	MS (18.9)	0	0	0	---	---	---	---	---	---
19	5	MS (9.4)	+	+	+	+	+	0	---	---	---
20	5	MS (4.7)	+	+	+	+	+	0	---	---	---
21	5	MS (1.9)	+	+	+	+	0	---	---	---	---

[1] FW = fresh water; MS = M.S. 222; UR = urethane. Concentration in p.p.m. is given in parentheses.

Results

Of five males which produced motile spermatozoa, two were used for tests of sperm viability in fresh water only, and three for tests in fresh water (control) and in dilutions of M.S. 222 and urethane (see table). In fresh water spermatozoa remained motile 90 to 210 seconds. In the presence of M.S. 222, spermatozoa remained motile less than 10 seconds at concentrations of 18.9, 37.7, and 75.5 p.p.m., and more than 10 seconds in four of seven tests at concentrations of less than 18.9 p.p.m. The tests were not adequate to establish tolerance limits more precisely. In 2,995 p.p.m. of urethane, spermatozoa remained motile for less than 10 seconds.

Conclusion

M.S. 222 at dilutions of 18.9 p.p.m., or stronger, made spermatozoa immotile. This dilution is one-fourth the strength of the solution generally recommended to anesthetize fish (2 grams in 7 gallons of water) and one-eighth of that used in the unsuccessful work at Marquette. Therefore, in spawn-taking, precautions must be taken to keep anesthetizing solutions of M.S. 222 from contacting the reproductive products. (The same applies to urethane, used at 2 ounces per 5 gallons of water.)

Acknowledgment

Messrs. Russell Robertson and Charles Shirtz of the State fish hatchery at Marquette, Michigan, assisted with this study.

The attention of fishery workers who plan to use urethane should be called to E. M. Wood's paper entitled "Urethane As a Carcinogen" (THE PROGRESSIVE FISH-CULTURIST 18 (3): 135-136).--The Editor.

A FRESH-WATER CLUPEID IN AN AQUARIUM

Clupeids endemic in Lake Tanganyika, Limnothrissa miodon Boulenger and Stolothrissa tanganicae Regan make up a very important part of the commercial fishery of this lake and rate as a significant item in the diet of many Africans. The success of the Institute in keeping a small number of Limnothrissa miodon alive in an aquarium for a year has provided an opportunity to study the growth, behavior, and feeding habits of this pelagic fish.

On June 18, 1959, a number of young fry were caught with big dip nets made of fine canvas and--without being taken from the water--were pushed into buckets and then emptied into a large container (about 150 liters). On the same day, about 40 of the fry were placed in an aquarium in the laboratory of the Institute at Uvira. Their mean length was 17.1 millimeters. They no longer showed the vitelline vesicle, but were still translucent and lacked scales.

The aquarium into which the fry were put had a capacity of 1,228 liters and was filled with surface water from Lake Tanganyika. The temperature of the water was held at 26.5° C. at first, but difficulties with the heating system caused it to cool slowly to 22° or 24° C. Aerated by an ordinary pump, the water was passed continuously through an external filter and was used in a closed circuit. Six small Cichlids endemic in the lake and two small crabs were put into the aquarium to scavenge on the bottom.

The food given to the fry was a paste made of finely crushed Stolothrissa meat. Small quantities of this were fed three times a day on every second day. The fish were fed only every second day because the water became turbid after the addition of the fish paste.

Only 12 individuals survived in captivity, and all of them were Limnothrissa miodon.

On May 10, 1960, one of the survivors was caught and examined. Its standard length was 45.6 millimeters; its weight was 1.10 grams. This was an average-sized fish, as the largest measured about 50 millimeters. The growth of Limnothrissa in the lake has not been studied, but in 1957 we showed that Stolothrissa in the lake reaches a length of 45 millimeters in 2.5 months. Nutrition was probably the limiting factor in the growth of the captive Limnothrissa. We could not supply the living shrimps of the pelagic species on which the fish usually thrive.

These pelagic fish did not filter water to get their food; they selected it, bit by bit. When food was placed in the water, feeding would begin with the short darts of individual fish from the school. Seconds later, however, the whole school would race into the descending cloud of particles, seizing them, one by one, until they neared the bottom. Then the school would resume its regular swimming movements.

The behavior of these fish in the aquarium was normal. When there was some light, even very dim, the fish swam about in dense, fast-moving schools. When in total darkness, the fish swam freely in all directions. They could not be found at rest at any time of the day or night.

---G. MARLIER, Institut pour la Recherche Scientifique en Afrique Centrale, Centre de Recherches du Tanganika, Uvira.

In Israel, where fish ponds may be adjacent to cottonfields, there have been instances of great mortality of fish as the result of aerial spraying of endrin, parathion, and dyphterex for the control of cotton-plant pests. For example, the entire populations of 2 ponds, amounting to 3.5 tons of marketable fish and more than 10,000 fingerlings, were killed after a plane that was spraying a field made its turns over the ponds.

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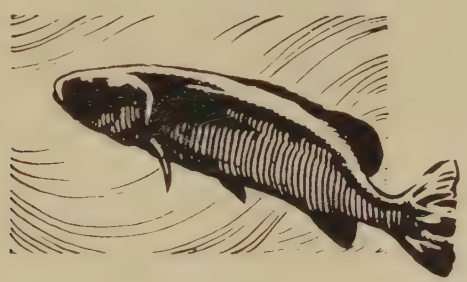

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
BUREAU OF SPORT FISHERIES AND WILDLIFE

INDEX, 1947 - 1956

This is an index to The Progressive Fish-Culturist during the 10-year period from 1947 through 1956. The first eight volumes of the periodical appeared between December 1934 and December 1941, after which publication was suspended for the duration of World War II. No cumulative index to the first eight volumes has been issued.

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